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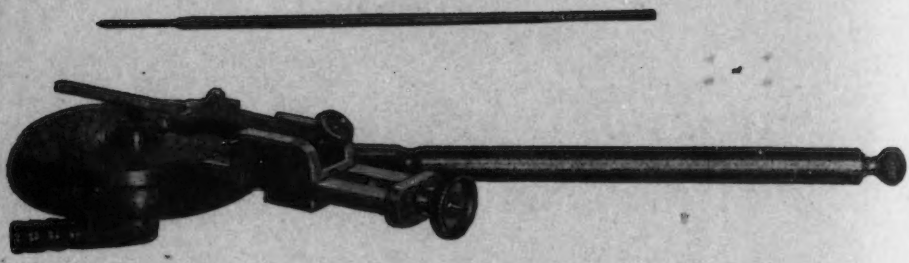

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THE VITAMIN A AND D CONTENT OF SOME MARGARINES

DOROTHY FETTER AND A. J. CARLSON

From the Physiological Laboratory of the University of Chicago

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It has long been recognized that the varieties of oleomargarines made from vegetable oils are inferior to butter in vitamin A content. However, some oleomargarines prepared from beef fat are reported not to show this deficiency (1), (2). Nevertheless, the laymen commonly regard all margarines as being inferior to butter. Margarines differ from butter in flavor. But the only value of food flavors in nutrition appears to be their influence on the digestive secretions and appetite, and this influence seems to be developed in the individual mainly on the basis of conditioned reflexes and may, accordingly, be associated with any flavor. Milk and milk products are only one of the many sources of vitamins in the diets of man. And among some people of normal stature milk or milk products form a part of the diet only in infancy. Hence it is clear that the vitamin A and D requirements for man can readily be met by other foods. The paucity of xerophthalmia cases in central Europe during the war shows us that even in times of serious deficiency of fats in the diet the human organism, especially adults on a mixed diet, can quite easily make up for the lack of milk fat factors by other foods.

The vitamin A and D content of two common margarines on the market were assayed for their vitamin A and D content in comparison with butter by the standard feeding tests on rats. The fat in one of these margarines was cocoanut oil, and that of the other oleo oil and lard. The composition of the latter is stated by the manufacturer[1] to be oleo oil, 41 per cent; neutral lard 33 per cent; moisture 11 per cent; vegetable oil 10 per cent; ash 5 per cent. The ingredients are churned in whole milk in such a way that nearly all of the solids in the milk go into the margarine. This probably is a factor in the vitamin A and D content of this margarine as the mixture may absorb the vitamins known to be present in skimmed milk.

[1] John F. Jelke Co.

In England various manufacturers have added vitamin concentrates to their products to make up for the deficiency of vitamin A in vegetable margarines (3). In order to determine if the margarines made largely from animal fats would be appreciably improved by similar additions, some of it was mixed with a "vitamin concentrate" in a ratio of 1 pound of the margarine to 7 minims of the concentrate, and some with cod liver oil in a ratio of 1 pound of the margarine to 15 minims cod liver oil. The company supplied the margarines to which cod liver oil and concentrate had been added. The oleo oil and the cocoanut margarines as well as the best creamery butter used in these experiments were purchased in first-class local retail shops.

Albino rats weighing from 50 to 70 grams were fed a synthetic diet in which no vitamin A was present, except that contained in the added fat. The diet used was that suggested by Palmer and Kennedy (4).

	per cent
Casein	18
McCollum's salt mixture, 185	3.9
Agar	2
Fat	5
Dextrin	to make 100

In addition 6 per cent of dried yeast was used to furnish sufficient vitamin B (6). The casein was devitaminized and rendered salt free according to the method of Palmer and Kennedy (7).

Thirty-six rats divided into six groups each consisting of six animals were put on the above diet, the fats being the only variable factor for each group. One group (curve *2*, fig. 1) received butter fat, and served as a standard for the comparison with the rats receiving the margarines. As a further check on vitamin A in the margarines, one group (curve *4*, fig. 1) was given a hydrogenated fat known to be deficient in this vitamin (8). The four remaining groups received as the fat content of the diet the other margarines tested. An additional group of six rats was fed the stock diet consisting of half bread, $\frac{3}{8}$ milk, $\frac{1}{8}$ meat. The purposes of this latter group were first to serve as a control for the growth of the rats on the synthetic diets, and second, to determine the amount of food necessary to insure good growth. Eight grams dry weight of the stock food per rat per day insures good growth according to the Donaldson average (9). The dry weight was roughly calculated by considering the bread as $33\frac{1}{3}$ per cent water, milk 87 per cent water, and meat 75 per cent water. Since this amount proved satisfactory, all the rats used were fed eight grams dry weight of the diet per day. Five to six grams daily have been reported as the usual intake of food for rats on a synthetic diet (10). The amount of food left in the dishes each day was considered to give an average record of the consumption.

Feeding with the above diets was continued over a period of 23 weeks. This experiment was repeated three times, twice with rats raised in the laboratory, and once with rats from the Wistar Institute. The results of the three sets of experiments were essentially the same. Records were taken of the growth of the rats as shown by the average weight of the six animals in each group. Also the general health of the rats as indicated by their activity and by the condition of the hair and eyes was watched. Since the animals were kept under uniform conditions, and since previous experiments showed that the fats of butter and at least some margarines have the same energy values in nutrition (11), (12) the variations in growth that could not be attributed to differences in food intake and sex were considered as due to the variations in the vitamin A content of the fats.

It should here be noted that the sex of the six animals of each group was always the same in order that pregnancy should not interfere with the weight records. The sex of the animals is an important consideration for the correct interpretation of the actual increase in weight. The male albino rat is normally larger than the female (13) and allowance must be made for this factor when judging the value of the food by the growth or weight of the rats.

Figure 1 shows the typical growth for each group of animals studied in one of the three experiments. Each curve represents average growth for the six animals of one group. A glance at the figure shows that the rats fed the stock diet were far superior in weight to any of those fed the synthetic diet. This is usually the case when the ingredients of the synthetic diet are highly purified (7).

The curve for group 2 shows that the rats which received butter as their fat allowance grew steadily for thirteen weeks with an average increase in weight of six grams a week. At the end of this time the growth curve flattens out, but shows a slight gain nevertheless. During the course of the experiment the rats were in good condition, being at all times active and sleek-coated.

The growth of rats on hydrogenated fat (no vitamin A) is shown in curve 4. It will be noted that the growth is distinctly subnormal. Symptoms of xerophthalmia appeared in about 50 per cent of the animals at the tenth week on the diet, and the eye condition became progressively worse. The condition of the coat became poor after six weeks, the rats losing hair in patches. Two rats died during the eleventh week of the experiment. Those left became progressively weaker, so that in the fourteenth week two were removed. One of these was fed the synthetic diet in which the fat content consisted of butter, while the other was given the diet with margarine made from animal fat as a source of vitamin A. After one week the rat which was fed butter gained 20 grams while the one on margarine gained 15 grams. The eye condition of both improved. These

changes showed immediate response to the vitamin. The two continued on the original diet died during the week, each weighing 65 grams, an increase of only 5 grams over the average weight of the set at the beginning of the experiment. In the repeat experiments, the longest duration of life of the rats fed hydrogenated fat was three months.

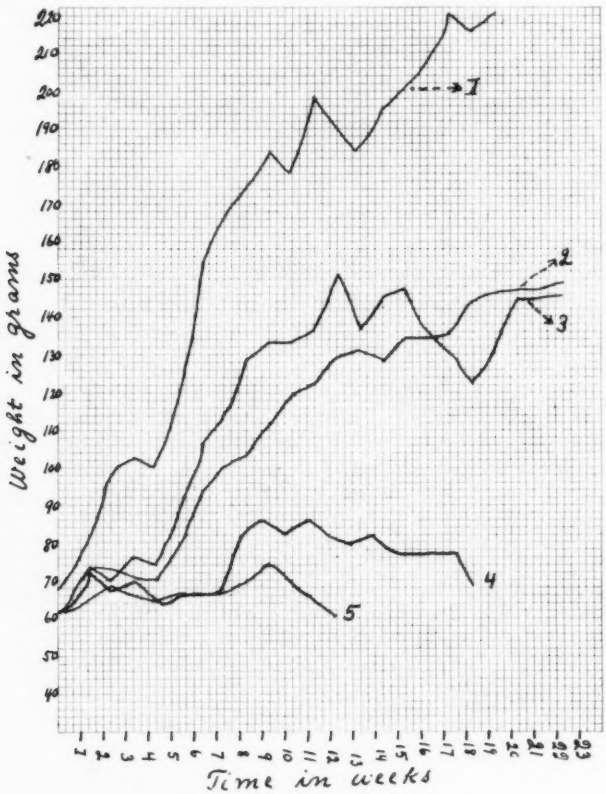

Fig. 1. Curves showing the growth of rats (6 in each group) on the purified synthetic diet with fat as the variable factor. *1*, control, on stock diet; *2*, butter fat; *3*, animal fat margarine; *4*, hydrogenated fat; *5*, cocoanut margarine.

The rats which received the margarines made from oleo oil showed growth at a rate of 10 grams a week for the first thirteen weeks of the experiment (curve *3*, fig. 1). Thereafter the curve flattened out. The curve was essentially like that for the rats which had been fed butter. However, in this experiment, a sudden decline in weight appeared at the eighteenth

week and the loss of weight continued for three weeks. Up to the period of the change, the rats were in good condition and ate their entire allowance of food. During the three weeks in which the rats lost weight, their hair condition became poor, they showed decreased activity, and ate very little. Two-thirds to three-fourths of the amount of food given was always left the next day. After three weeks, however, the rats started to eat again, the hair condition improved, and the animals regained their previous weight. The reason for the sudden decline could not be determined. It

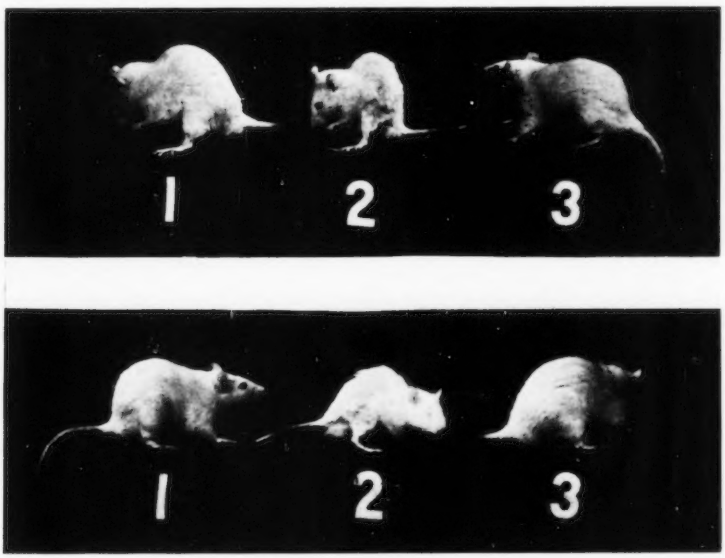

Fig. 2. *1*, rats on the vitamin A free diet + 5 per cent oleo oil-lard-milk margarine for 22 weeks; *2*, rats on the vitamin A free diet + 6 per cent hydrogenated fat for 12 weeks; *3*, rats on the vitamin A free diet + 5 per cent butter for 22 weeks. Showing the practical identity in vitamin A content of this margarine and standard butter.

could not have been due to a lack of vitamin A because if the diet had been deficient in this substance, the rats could not have grown as they did during the first eighteen weeks of the experiment. Also, the improvement noted later shows that the food could not have been the source of the trouble. This peculiar setback did not appear in the other two experiments. The fact that for the first eighteen weeks of this experiment the weight of the rats fed this margarine exceeded that of the rats fed butter must not be taken as an indication that the margarine was superior to butter in vitamin A content. As was stated above, the weight of the

male normally exceeds that of the female, and in the experiment quoted the rats fed the margarines were males while those fed butter were females. In another experiment in which the rats fed butter were males and those fed margarines were females, the weight relation was inversed and those fed butter were about 20 grams heavier than the others. Thus an equality in the two foods in regard to vitamin A content is indicated.

For the first twelve weeks of one experiment, the rats fed the animal fat margarine plus a trace of cod liver oil showed approximately the same rate of growth as those fed butter. The curve shows a decrease in weight after this period, which is probably due to the fact that the rats did not eat all the food given them from this time on. In the other two experiments, the rats of this group continued to eat all their food during the experiment and no decrease in weight was observed. The weights of these rats ran parallel to the weights of those fed butter, provided the sex was the same. The evidence indicates that the margarines to which a trace of cod liver oil had been added showed no superiority over the margarine without the addition.

The rats receiving as fat the oleo oil margarine with a trace of "vitamin concentrate" gained about 60 grams in the first two months, and then ceased to gain appreciably. There was no improvement noted in the condition of these rats over those fed the margarine now on the market.

The rats which received the cocoanut oil margarine as their source of fat showed very little growth (curve 5, fig.1). In all ways they appeared about the same as the rats which were fed the hydrogenated fat. Xerophthalmia appeared in two months. After eleven weeks on this diet, the animals died. The results show the extreme deficiency of vitamin A in this vegetable margarine.

The method for testing any food for the content of its antirachitic factor is completely described by McCollum, Simmonds, Shipley and Park (14). Their procedure was followed throughout the experiment. The experiment was repeated twice, first with albino rats weighing from 50 to 60 grams, and second with rats weighing from 70 to 100 grams. Twenty-five rats were used each time. These were divided into five groups of five rats each. All the groups of rats were fed McCollum's rachitic diet, 3134 (14). The rats which weighed from 50 to 60 grams were kept on the diet for five weeks, while those which weighed from 70 to 100 grams remained on it for six weeks because of their greater resistance to the disease. At the end of this period, the rats showed symptoms of rickets. One rat of each group was killed at this time, and the fact that they had rickets was verified by the line test (14).

After the rats had become rachitic they were fed McCollum's diet, 3143, plus 5 per cent of the fat to be investigated. The fats were freed from salts according to the method described by Palmer and Kennedy (7).

This is necessary in order to make sure that any improvement in the calcification of the bones of the animals on the diet is due not to a change in the ratio of calcium to phosphorus, but to the anti-rachitic factor in the fat.

Group I received butter as the fat content of the diet; group II received the animal fat margarine bought in open market; and group III received the animal fat margarine with the trace of cod liver oil added. As a further

TABLE 1

GROUP	RAT NUMBER	CURATIVE ELEMENT ADDED TO DIET	WEIGHT WHILE ON CURATIVE DIET			LINE TEST
			April 28	May 5	May 8	
1	1	Butter	75	76	75	+ heal
	2	Butter	70	72	76	+ heal
	3	Butter	97	100	100	0 heal
	4	Butter	78	75	80	0 heal
2	1	Animal fat margarine	101	104	100	++ heal
	2	Animal fat margarine	88	88	88	++ heal
	3	Animal fat margarine	88	90	90	+++ heal
	4	Animal fat margarine	58	58	54	+++ heal
3	1	Animal fat margarine plus cod liver oil	80	88	84	+++ heal
	2	Animal fat margarine plus cod liver oil	72	70	70	+++ heal
	3	Animal fat margarine plus cod liver oil	113	112	112	+ heal
	4	Animal fat margarine plus cod liver oil	112	112	112	++ heal
4	1	Cod liver oil	70	72	76	+++ heal
	2	Cod liver oil	75	76	75	+++ heal
	3	Cod liver oil	60	58	54	+++ heal
	4	Cod liver oil	50	42	43	+++ heal
5	1	No addition to diet	90	90	88	0 heal
	2	No addition to diet	70	70	70	0 heal
	3	No addition to diet	68	60	64	0 heal
	4	No addition to diet	45	42	40	0 heal

control on these fats, group IV received 5 per cent cod liver oil. Group V received McCollum's diet 3143 without any additional food throughout the experiment. The last group served as an additional control.

After a period of ten days on the diet containing the food under investigation, the rats were killed and subjected to the line test. This test will show healing of rickets in four fairly distinct stages known as plus 1, plus 2, plus 3, and plus 4.

The rats on the completely rachitic diet (group V), which had either maintained their weight or lost 4 to 5 grams, were found to have severe rickets at the end of that period as shown by the complete lack of calcification of the epiphyseal disc of cartilage of the tibia. Accordingly such changes in calcification as were found in rats fed the rachitic diet plus the fats were considered as due to vitamin D, provided that the rats had either gained weight, maintained it, or lost not more than 4 grams during the ten days of the test diet.

The rats on the control diet with cod liver oil showed a plus 3 cure. This grade of cure is taken as the standard since cod liver oil is known to have a high content of vitamin D. The rats which received the butter showed a plus 1 cure in about 50 per cent of the cases, and rickets was present in the other 50 per cent. This indicates that butter has little of the antirachitic factor. Previous work also indicates that milk products have a low vitamin D content. The rats which were fed the oleo oil margarine showed a variation of plus 2 cures in 50 per cent of the cases, and plus 3 cures in the other 50 per cent. This indicates that this margarine is relatively high in vitamin D. The rats which received the margarine to which a trace of cod liver oil had been added showed a plus 1 to a plus 3 cure.

Table 1 shows the cure of rickets as revealed by the line test for four rats of each of the five groups. Weight records are given for the period during which the rats received the foods under investigation.

SUMMARY

As tested on rats the animal fat margarine churned in whole milk is equal to butter in vitamin A content. The margarine with cocoanut oil as the fat content is very deficient in vitamin A.

As tested by the cure of rickets in rats the oleo-oil-lard-milk margarine studied is superior to butter in vitamin D content.

BIBLIOGRAPHY

(1) Halliburton and Drummond. Journ. Physiol., 1917, li, 235.
(2) Osborn and Mendel. Journ. Biol. Chem., 1915, xx, 379.
(3) Coward. The Lancet, 1928, ii, 726.
(4) Palmer and Kennedy. Journ. Biol. Chem., 1922, iv, 217.
(5) McCollum, Simmonds and Pitz. Journ. Biol. Chem., 1916, xxv, 123.
(6) Chick and Roscoe. Biochem. Journ., 1927, xxi, 698.
(7) Palmer and Kennedy. Journ. Biol. Chem., 1927, lxxv, 615.
(8) McCollum, Simmonds and Pitz. This Journal, 1916, xli, 361.
(9) Donaldson. The rat. 2nd ed. 1924, Chap. 5.
(10) Funk. The Nation's Health, 1922, iv, 393.
(11) Hulbgren and Landergren. Pflüger's Arch., 1901, lxxxiii, 609.
(12) 19th Annual Report, Mass. State Board of Health.
(13) Donaldson. The rat. 2nd ed., 1924, 276.
(14) McCollum, Simmonds, Shipley and Parks. Journ. Biol. Chem., 1922, li, 41.
(15) Funk. The Nation's Health, 1922, iv, 393.

MUSCULAR TRAINING

II. The Effect of Training on the Recovery Period Following Severe Muscular Exercise

C. GEMMILL, W. BOOTH, J. DETRICK and H. SCHIEBEL

From the Department of Physiology, School of Medicine, The Johns Hopkins University

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In a former paper (Gemmill, Booth and Pocock, 1930) the effect of daily repetition of the same amount of light muscular work on a bicycle was studied on three subjects and a decrease was observed in the total ventilation and the pulse rate as the training progressed. The total metabolism during work also showed a slight decrease. In order to see if the changes would be more marked under severe exercise, we have made similar experiments on subjects training to running.

Method. During the spring of 1929 an attempt was made to study the effect of training on track men of this university. Seven men on the track team and seven non-athletes for controls were selected at the start of the season and their metabolism for running seventy yards was obtained using the method of Sargent (1926). It was planned to repeat these determinations for the same distance and time at the end of the track season. However, in these experiments it was very difficult to control adequately the men and the external conditions. The subjects were not accustomed to metabolic experiments, not in the basal state, only one determination on each subject could be obtained; external temperature was markedly different for the two groups of experiments, one made in March and the other in May in an open field. Differences in the track and the fact that only two of the seven men of the track squad measured were still on the team at the end of the season made us decide to do similar experiments under laboratory conditions where better control could be had over the external conditions and the subjects. For this purpose a treadmill[1] was obtained very similar to the one that Benedict and Murschhauser (1915) used for walking experiments. Our mill has a range of speed from 225′ to 1800′ per minute, adequate for any type of walking or running experiments. For the protection of the runner, a heavy canvas belt was strapped around his chest and fastened by ropes to an overhead frame.

[1] The authors wish to thank the Helen Hartley Jenkins Fund for Medical Research for their financial aid in securing the treadmill.

The subjects were in good health, had not done very strenuous work for some years and were accustomed to metabolic experimentation. The individual data for the two subjects were as follows: C. G., age 28, height 180 cm., weight 82.5 kilos; W. B., age 25, height 176 cm., weight 68.8 kilos. The weights given were obtained at the start of the experiments.

On the day of an experiment the subjects came to the laboratory without breakfast, dressed in track clothes, rested one hour on a bed beside the treadmill, then breathed through the valve system for ten minutes to wash out the dead space in the connections and spirometers. A few of the experiments were done in the evening, the subject going without his dinner. In the majority of the experiments, two ten-minute basal samples were collected, in two experiments one ten-minute sample was taken. The subject then climbed on the treadmill, the mill was started by an assistant pulling the starting lever as rapidly as possible up to a fixed mark on the control box and then stopped the mill ten seconds later by turning the control lever back. The speed of running while the current was on was thirteen miles per hour, this speed being reached during the second or third revolution of the belt, after ten to fifteen yards of running. During the ten-second interval while the current was on the runner ran sixty-three yards. After the current was turned off the mill quickly slowed down, stopping five to seven seconds later. In this period the mill made five additional revolutions; so as the runner was slowing down, he ran an additional twenty-nine yards. The subject then returned to the bed, adjusted the mouth piece and nose clip and breathed into the spirometers. During the run the subject attempted to hold his breath and was generally able to do so up to the time the mouth piece was adjusted on his return to the bed. The run, the stopping of the treadmill and the return to the bed took about thirty seconds. This corresponded to the run on the track, the "pull-up" and the adjustment of the nose clip and mouth piece in Sargent's (1926) experiments.

Two eighty-liter spirometers were used, connected by suitable valves, so that by turning one valve, the expired air passed into one spirometer and the other spirometer was shut off. In this manner a continuous collection of the expired air was made for sixty to seventy minutes following the run with the subject resting in bed. Samples of gas were collected over mercury and analyzed in a modified Haldane apparatus. On each day before any analyses of the expired air were made, a sample of atmospheric air was analyzed, checking for the combined carbon dioxide and oxygen 20.96 per cent $\pm$ 0.03 per cent.

Breathing through a mouth valve for sixty minutes causes some discomfort to the subject so a control experiment was made to see if this discomfort affected the metabolism. The subject, C. G., resting on a bed in a basal condition breathed through the valve for sixty-six minutes. Dur-

ing this time seven continuous samples were taken and the results given in table 1. The oxygen consumption and the R.Q. remained practically constant throughout this period.

With the rapid changes in the oxygen and carbon dioxide content of the expired air in the earlier part of recovery, it is very important to get complete mixing in the spirometers. In order to see if complete mixing of the gases in the spirometers was being obtained, in several experiments two samples of gas were taken of the first collection after work, one sample when the spirometer was pushed one quarter of the way down and the other sample when it was three-quarters exhausted. These two samples in each case agreed within the limits of the analyses (± 0.03 per cent) showing that complete mixing of the gases was obtained in the spirometers.

TABLE 1

Summary of control of basal metabolism

Subject C. G.

TIME	O_2	CO_2	VENTILATION (CORRECTED TO 0° AND 760 MM.)	R.Q.	PULSE	RESPIRATION
	cc. per minute	*cc. per minute*	*l. per minute*		*per minute*	*per minute*
0.00– 9.55	257	199	4.50	0.77	58	6
9.55–19.10	261	209	4.74	0.80	54	6
19.10–28.65	259	202	4.64	0.78	58	7
28.65–38.20	262	206	4.67	0.79	54	6
38.20–47.75	265	211	4.80	0.80	54	8
47.75–57.30	269	209	4.85	0.78	56	6
57.30–66.85	263	202	4.61	0.77	54	7

The values for the time are given in minutes and decimal parts of minutes.

During the recovery period pulse rates, respiration rates and blood pressures were taken at two or three minute intervals. The pulse rate was obtained by radial palpation, respiration by direct counting, and the blood pressure by the ausculatory method. The temperature and humidity of the room were observed each day of the experiment. The weights of the subject were also recorded.

The speed of the mill was controlled by calibrating the treadmill for a half-minute run before the experiment and by recording the total number of revolutions during each experiment. The number of revolutions for each experiment did vary slightly from experiment to experiment yet no correlation could be observed between these changes and the oxygen consumption.

Four experiments were made on C. G. and three on W. B. before training on the track was undertaken. This training consisted of running from two to three miles each day in addition to practicing short dashes. When

the subjects started they were unable to run more than one-quarter of a mile without stopping, so in order to cover the required distance they ran one lap of the track and then walked one. Gradually as the initial aches and pains wore off, greater distances were attempted and the final procedure for the last three weeks was to run one mile, then walk and run alter-

TABLE 2

Summary of metabolism before and after training

Subject C. G.

EXPERIMENT	DATE	RESTING—LITERS PER MINUTE		RECOVERY				R.Q.			TIME OF RUN	TOTAL REVOLUTIONS	RELATIVE HUMIDITY	TEMPERATURE OF ROOM
				Gross liters per 60 minutes		Net liters per 60 minutes								
		CO_2	O_2	CO_2	O_2	CO_2	O_2	Resting	Gross	Net				
Before training														
											seconds		*per cent*	°C.
1	April 4	0.186	0.285	18.994	23.564	7.834	6.464	0.65	0.81	1.21	$9\frac{3}{5}$	47	32	25
2	April 11	0.207	0.267					0.78						
		0.200	0.263	20.091	20.847	7.881	4.947	0.76	0.96	1.58	$10\frac{1}{5}$	52	41	24.4
3	April 12	0.188	0.275					0.68						
		0.195	0.267	18.586	20.956	7.096	4.696	0.73	0.89	1.51	10	52	37	25
4*	April 22	0.214	0.289					0.74						
		0.217	0.290	19.675	23.789	6.745	6.419	0.75	0.83	1.05	10	48	40.5	26.2
During training														
5	May 6	0.202	0.268					0.75						
		0.221	0.270	19.105	21.222	6.415	5.082	0.82	0.90	1.26	10	45	38	26.9
6	May 20	0.201	0.248					0.81						
		0.206	0.257	17.796	20.692	5.586	5.542	0.80	0.86	1.01	10	47	55	24.4
7	May 26	0.216	0.280					0.77						
		0.219	0.293	20.429	22.887	7.379	5.727	0.75	0.89	1.29	10	54	36	25.5

nate laps totaling from three to four miles per day. In addition short dashes were practiced to be comparable to the work on the mill and long walks were taken in the afternoon, on some days as much as eighteen miles were covered in addition to the morning run. The object of the walking was to build up the leg muscles generally and the running to build up the particular systems needed when running on the treadmill.

After two weeks of this work another laboratory experiment was made and at the end of a month of training two additional experiments on each

TABLE 3

Summary of metabolism before and after training

Subject W. B.

EXPERIMENT	DATE	RESTING—LITERS PER MINUTE		RECOVERY				R.Q.			TIME OF RUN	TOTAL REVOLUTIONS	RELATIVE HUMIDITY	TEMPERATURE OF ROOM
				Gross liters per 60 minutes		Net liters per 60 minutes								
		CO_2	O_2	CO_2	O_2	CO_2	O_2	Resting	Gross	Net				
Before training														
											seconds		*per cent*	°C.
1	April 8	0.181	0.232					0.78						
		0.193	0.222	19.138	20.078	7.918	6.458	0.86	0.95	1.24	10	56	28	26.1
2	April 10	0.189	0.241					0.78						
		0.183	0.235	16.673	18.233	5.513	3.953	0.78	0.92	1.39	10	56	24	25.5
3*	April 23	0.185	0.241					0.77						
		0.196	0.245	16.310	19.365	4.880	4.785	0.80	0.84	1.02	10	47	35	25.0
During training														
4	May 8	0.205	0.261					0.75						
		0.206	0.251	15.335	18.167	3.005	2.807	0.82	0.84	1.07	10		74	28.8
5	May 21	0.198	0.254					0.78						
				16.057	18.872	4.177	3.632		0.85	1.15	10	46	48	25.0
6	May 22	0.210	0.255					0.82						
		0.209	0.259	15.690	18.467	3.120	3.047	0.81	0.81	1.02	10	47	53	25.5

* A continuous sample of sixty minutes was not taken in experiment 4 on C. G. and experiment 3 on W. B., so these experiments are not strictly comparable with the other experiments.

subject were carried out. During the month of training the subjects missed four days of training due to adverse weather conditions.

RESULTS: The results[2] are given in tables 2, 3, 4, 5.

Metabolism: In order to compare the various experiments, all the values

[2] In order to conserve space, the complete data on metabolism, R. Q., ventilation, respiration per minute, pulse rate, blood pressure and the method of calculation of the logistic function have been omitted from the paper. Mimeograph copies of these data have been made and will be sent to anyone interested on request.

for the total metabolism have been reduced to a sixty minute recovery period. It was soon found in the earlier experiments that even with a recovery period of sixty minutes, the oxygen consumption had not returned to the base line but was still ten to thirty cubic centimeters above the basal level. In order to see if the metabolism became lower during the

TABLE 4

Total ventilation for sixty-minute recovery period

SUBJECT C. G.			SUBJECT W. B.		
Date	Gross	Net	Date	Gross	Net
		Before training			
	liters	*liters*		*liters*	*liters*
April 4	580.57	261.97	April 8	503.01	166.41
April 11	552.50	240.50	April 10	454.58	144.38
April 12	501.37	224.17	April 23	435.77	127.97
April 22	571.55	235.55			
		Training started April 25			
May 6	583.21	246.61	May 8	429.25	64.45
May 20	505.19	190.79	May 21	430.29	105.09
May 26	513.26	196.46	May 22	399.90	43.50

TABLE 5

Summary of values of C and r in logistic function $y = \frac{K}{1 - Ce^{rt}}$ *for metabolism and ventilation*

EXPERIMENT		C. G.				W. B.			
		Metabolism		Ventilation		Metabolism		Ventilation	
		C	*r*	*C*	*r*	*C*	*r*	*C*	*r*
Before training	1	0.9577	−0.0062	0.9880	−0.0041	0.9696	−0.0053	0.9804	−0.0205
	2	0.9226	−0.0115	0.9863	−0.0076	0.9303	−0.015	0.9590	−0.0251
	3	0.9215	−0.0133	0.9831	−0.0064	0.9230	−0.0099	0.9656	−0.0189
	4	0.9554	−0.0140	0.9829	−0.0081				
During training	5	0.9484	−0.0115	0.9890	−0.0041	0.8913	−0.023	0.9881	−0.0530
	6	0.9423	−0.0105	0.9772	−0.0076	0.9484	−0.009	0.9495	−0.0117
	7	0.9533	−0.0073	0.9856	−0.014	0.9333	−0.017	0.9462	−0.0989

second hour, one experiment on each subject was made in which continuous samples were taken for forty-five minutes and then two or three discontinuous samples were taken during the second hour. In experiment 4, on C. G., the determination at the end of the second hour was practically the same as at the end of the forty-five minute period, yet it was still

twelve cubic centimeters above the base line. The experiment on W. B., number three, showed a greater drop between the sample taken at the forty-five minute period and the ninety-eight minute period yet the final sample was still above the base line. With these slight changes in metabolism as the oxygen consumption approached the base line, it was practically impossible to tell exactly when the metabolism had returned to normal so an arbitrary recovery period of sixty minutes was selected in order to compare results. This slow return of the metabolism to normal has been noticed by many observers, both for long continued exercise (see review of literature by Benedict, 1913, p. 163) and for severe exercise of short duration (Hill and Lupton, 1923).

The question of comparing oxygen usage during and following muscular exercise involves a base line. This question was discussed in part one of this series, and for light muscular exercise it was concluded that the total oxygen used during work was the best value to take for comparable experiments. It was also the preferable value for the study of the recovery period. In order to get the net or the excess metabolism due to the work in our experiments, it was necessary to multiply the basal value by sixty and to substract that value from the total oxygen used. Any slight variation in the basal metabolism when multiplied by sixty makes a large difference to be subtracted from the total. Then the basal base line may shift during and after work, for Benedict (1913) has pointed out that work is a stimulus to the basal metabolism and the value for the metabolism for the production of external effective work may change with the conditions under which the work is done. With a changing basal level and a slow return to normal following work it is not correct to take the basal value before work as a base line or even the value at any arbitrary point of the recovery period as Hill, Long and Lupton have suggested (1925) in order to get the excess metabolism due to work. With a changing base line the term "excess" metabolism has no meaning. The values for both the gross and the net metabolism are given, the gross metabolism is used to compare our experiments with each other and the net values with those of other observers.

In tables 2 and 3 the results of the metabolic experiments are given. In C. G. before training, the gross metabolism varied from 20.8 liters to 23.8 liters for the sixty minute recovery period, and during training it varied from 20.7 liters to 22.9 liters. In W. B. before training the metabolism ranged from 18.2 liters to 20.1 liters and during training from 18.2 liters to 18.9 liters. The results during training would give a lower average than before training, yet with the metabolism of one experiment before training being as low as during training in each case, the change in metabolism for doing a specific amount of work before and after training was within the limits of variation of our experiments. The value for the

metabolism of the recovery period for the first experiment on each subject was high compared with the others; if only two experiments had been made one before and one at the end of training it would appear that the metabolism in each case had decreased, for in C. G. on April 4 the gross recovery period was 23.6 liters and on May 26 it was 22.9 liters. In W. B. on April 8 the recovery period was 20.08 liters and on May 22 it was 18.5 liters. The net recovery showed similar changes. A net recovery period of five liters for a ten second run corresponds to the more strenuous types of exercise studied by Hill (1923) and represents 14 horsepower uncorrected for start and "pull up," approximately similar to a corrected value of 13 horsepower used by Fenn (1930) in his studies of runners on outdoor tracks. The gross and net R. Q. showed no definite change.

Ventilation. The gross and net ventilation for the sixty minute period before and during training is given in table 4. The values for the respiratory rates as well as for pulse rates and blood pressure were taken from two to three minute intervals. In W. B. all the values for the total and net ventilation were less during training than before, for C. G. the average was lower during training yet one result before training was as low as any of the results during training. In C. G., comparing the rate of respiration after training with the results before training we find that the return to normal was quicker. The rate at the end of the twenty minute period was practically that of the basal value. The values for the first minute during training were the same as at the start of training. In W. B. the values at the beginning of the recovery period during training were lower than before training yet the return to normal was practically the same before and during training.

Pulse rates and blood pressure. The changes in the pulse rate during the recovery period showed a greater change during the period before actual training was started. On April 11 in C. G. the pulse rate did not rise as high as in the first experiment and the return to normal was much quicker, although in no experiment on C. G. did the pulse rate return to its basal value. In W. B. similar changes were observed in the period before training. The pulse rate of W. B. approached more nearly the basal value at the end of the sixty minute period than that of C. G.

No marked changes were observed in the height of the rise or the return to normal in the blood pressures of W. B. and C. G. before and during training. W. B. showed a drop in the diastolic level of the blood pressure during the earlier stages of recovery.

C. G. lost 2.5 kgm. in weight during the training, W. B.'s weight remained constant.

Discussion. Both subjects, as training progressed, noticed that they were able to run faster and for longer distances, and found that the running experiments on the treadmill became easier as training proceeded.

W. B. improved during training much faster than C. G., being able to outdistance C. G. without any difficulty.

From our experiments the greatest changes that contributed to this increased ability to do a certain amount of work was that of total ventilation. In W. B. all the values for total ventilation during the training period were lower than before training, but in C. G. there was one value during the preliminary experiments that was as low as values during training. The rate of respiration decreased in W. B. Suggestions as to the cause of these changes were given in part one (Gemmill, Booth and Pocock, 1930) of this series and further work will be necessary to determine the actual mechanisms involved.

In our former work we found a slight decrease in the metabolism and in this series of experiments the changes during training were of the same order as the variations in the experiments. This probably signifies that the amount and time course of the oxidation of lactic acid produced in short bouts of violent muscular exercise is not affected by training. Some observers (see table 7, Gemmill, Booth and Pocock, 1930) have reported large changes in metabolism and muscular efficiency during training. Does the actual efficiency of any muscle increase during training, the efficiency in the physical sense of the ratio of output of energy to the input and not merely the ability to do external work? The difficulty of determining this fact is that it is difficult to get an accurate measure of the total external work, i.e., effective work plus extraneous movements. In the experiments on the bicycle and treadmill doing comparatively simple types of muscular motion where extraneous movements were at a minimum the efficiency of the groups of muscles used in the work has been practically the same before and after the period of training.

We also found in light muscular work a decrease in the pulse rate and quicker return to normal. This was not so marked in this series of experiments.

It was surprising how few significant changes were observed when both subjects felt that they were doing the same amount of work more easily. It may be said that the subjects did not train long enough, but running two to three miles a day for a month and walking long distances during this time should bring about some measurable change.

Various attempts have been made to represent the course of the oxygen consumption during the recovery period graphically. Hill (1925) plotted the oxygen consumption for the separate periods in terms of oxygen utilization per minute against time and suggested that the curves were exponential in character and were analogous to the slow return of an over damped mechanical system to its position of equilibrium but did not attempt to fit his suggested formula to the curve. Simonson (1927) converted the oxygen used during recovery into a curve so that any point on the curve repre-

sented the oxygen yet to be used, i.e., the amount of lactic acid that was to be oxidized. These two methods have been discussed by Hebestreit (1929) who used a negative exponential function similar to Simonson to express mathematically the course of oxidation during the recovery period. At the suggestion of Doctor Berkson, the logistic function (Reed and Berkson, 1929) was applied to the data after converting it into the curve relating

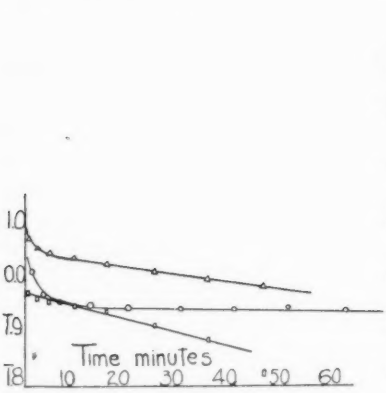

Fig. 1

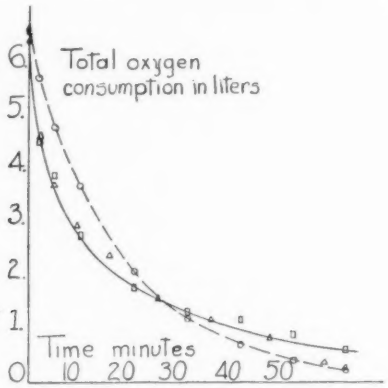

Fig. 2

Fig. 1. □ Logistic function: $\log \frac{y-k}{y}$ plotted against time where y is the total oxygen yet to be used at time t and k is basal metabolism.

△ Simonson's (1927) method: $\log y$ plotted against time.

○ Hill's (1925) method: $\log y$ plotted against time.

Fig. 2. ○ Calculated values for oxygen consumption from equation $y = 6.546\ e^{-0.0552t}$ (Simonson's method, 1927) for data from experiment 1 on C. G. (broken line).

□ Calculated values for oxygen consumption from equation

$$y = \frac{0.285}{1 - 0.9577\ e^{-0.0062t}}$$ for experiment 1 on C. G.

△ Actual values for oxygen consumption as determined from data of experiment 1 on C. G.

the total oxygen to be oxidized with time, and a very close correspondence was found during the earlier part of the experiment. During the last part of the recovery period the curve drops off, probably suggestive that another reaction may enter at this point. In figure 1 the method of Hill, Simonson and the present method are compared taking the data from Experiment 1 on C. G. The present method approaches a straight line more closely than the other two.

The logistic function is $y = \frac{k}{1 - Ce^{rt}}$ where, in our case, y is the amount of oxygen yet to be used at any time, t, k is basal metabolism, C and r being constants for any given experiment.

In logarithmic form the equation is

$$\log \frac{y - k}{y} - \log C = \frac{rt}{2.3}$$

so by plotting $\log \frac{y - k}{y}$ against time the value of the slope r of the line and antilog C of the y intercept of this line may be obtained.

The correspondence of the observed and calculated values are given in figure 2, and the table of values for all experiments is given in table 5.

This function signifies that, assuming lactic acid is the substance oxidized, the rate change of the lactic acid with time is proportional to the product of two factors, y and y-k suggesting a bimolecular reaction. A similar suggestion was made by Hartree and Hill (1922) from a study of the heat production during the recovery process in isolated muscle following tetanus. In all of the experiments a slight s-shaped bending from the straight line is noticed when the value of $\log \frac{y - k}{y}$ and t are plotted. This suggests that the function may be a more general one and rate of change may be proportional to more than two factors; yet for comparative purposes and the fact that we get a close check on the observed and calculated values we have taken the above equation.

A study of the values of C and r in table 5 for ventilation and metabolism showed that they do not group themselves into two distinct groups before and after training. Theoretically, the use of this equation would give an ideal method for comparing individuals as the value of C gives a measure of the total oxygen consumption and the value of r gives the rate of decrease to normal, the two values being an index of the subject's reaction to a particular amount of strenuous work. An extensive study should be made under similar conditions on a larger group of athletes to see if an adequate test might not be developed for training, especially on the basis of respiration which we have shown does change during training. As yet, we have no adequate test for judging the condition of a subject for strenuous exercise.

The only experiments of this type that we have found in the literature are those of Simonson (1927) and Liebenow (1928). Simonson studied the recovery period following light muscular work and by using the formula

$$Rk = \frac{l}{t} ln \frac{\text{cal. } A}{\text{cal. } t}$$

found that the *Rk* value was constant for a given individual but increased during training using (arbitrary) times $t = 0$ and $t = 3$ for the calculation of the *Rk*. He also found a decreased oxygen debt during training. As his oxygen debts were only from 900 to 1200 cc., his experiments were not strictly comparable to those with oxygen debts of 5 liters, training to exercise of much greater intensity.

Liebenow studied the recovery period after climbing stairs and found that the excess oxygen consumption and the time of the recovery period decreased in their experiments. In one subject the excess oxygen consumption decreased from 8.057 liters to 2.767 liters after five experiments. He concluded that training decreases the oxygen used for doing a particular amount of work as he stated, that the training of coördination in such daily and accustomed work could only play a very subordinate rôle. We may be accustomed to going up and downstairs but not at such a rate of speed to cause a recovery oxygen debt of eight liters, equivalent to a 225 yard dash in 23.4 seconds (Hill and Lupton, 1923). Climbing stairs at that rate must demand many new coördinations not used in every day work. In table 3 of Liebenow's paper the excess oxygen used is given and the results are comparable to ours before training, the first experiment giving a high value and then dropping to a new and fairly constant level, in subject *K* changing from 8.470 liters to 5.701 liters in the second experiment five days later. It is doubtful whether actual chemical changes in the muscles themselves can take place to cause this great change in the oxygen used in two experiments. A better explanation is better coördination in the later experiments, the total mass of muscle used being less in the second experiment which would decrease the oxygen used.

SUMMARY

A study was made of the effect on the metabolism, ventilation, respiratory and pulse rates and blood pressure during the recovery period following severe exercise on a treadmill in two subjects before and during a course of training to running.

The metabolism, blood pressure, pulse rates and gross and net R. Q. for the recovery period to the standard work did not change during the training.

The total ventilation of W. B. showed a decrease in amount, for C. G. one result before training was as low as any of the results during training. There was an increased rate of return to normal in both subjects.

The number of respirations in W. B. during the earlier portion of the recovery period was less during training than before. In C. G. the rate of return to normal was increased by training.

The course of the recovery period can be expressed in terms of the logistic function.

BIBLIOGRAPHY

BENEDICT, F. 1913. Carnegie Inst. of Wash. Publ. 187, 173–176.
BENEDICT, F. AND H. MURSCHHAUSER. 1915. Carnegie Inst. of Wash. Publ. 231.
FENN, W. O. 1930. This Journal, xcii, 583.
GEMMILL, C., W. BOOTH AND B. POCOCK. 1930. This Journal, xcii, 253.
HARTREE, W. AND A. V. HILL. 1922. Journ. Physiol., lvi, 367.
HEBESTREIT, H. 1929. Pflüger's Arch., ccxxii, 738.
HILL, A. V., C. N. H. LONG AND H. LUPTON. 1925. Proc. Roy. Series B, xcvii, 96.
HILL, A. V. AND H. LUPTON. 1923. Quart. Journ. Med., xvi, 135.
LIEBENOW, R. 1928. Zeitschr. f. d. gesammt. exper. med., lix, 49.
REED, J. R. AND J. BERKSON. 1929. Journ. Physical Chem., xxxiii, 760.
SARGENT, R. M. 1926. Proc. Roy. Soc. Lond. Series B, c, 10.
SIMONSON, E. 1927. Pflüger's Arch., ccxv, 716, 743, 752.

THE ACTION OF TESTIS HORMONE IN CORRECTING CHANGES INDUCED IN THE RAT PROSTATE AND SEMINAL VESICLES BY VITAMINE B DEFICIENCY OR PARTIAL INANITION[1]

CARL R. MOORE AND L. T. SAMUELS

From the Department of Zoölogy and Department of Physiological Chemistry, The University of Chicago

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The effects of various vitamine deficiencies upon the mammalian reproductive system, particularly upon the testis, has received considerable attention (1 to 11) but no adequate study has been made of the effect upon the hormone secreting capacity of this organ. Perhaps this has been due to the lack of adequate methods for determining hormone production. Through the work of Moore and his associates several different methods have been developed that are adequate for studying the hormone secretion by the testis during short periods of time (12 to 21). Among others, the cytology of the prostate gland and the seminal vesicle of the rat have proved to be very dependable hormone test methods applicable over periods of a few days to a week and longer.

Incidental to other studies carried on by Samuels in which rats were kept on a diet deficient in the antineuritic factor of vitamine B it was thought advisable to examine the reproductive system of the male animals. This study, carried out by Moore, proved of exceptional interest in that the testicles of such rats were found to be essentially normal histologically but the prostate gland and seminal vesicles were as castrate in type as if the testicles had been removed some weeks previous. It was obvious either that the testis was not secreting hormone or that the male reproductive accessories were incapable of reacting to the hormone because of the general decline induced by the deficient diet.

In order to investigate the basic cause for this interesting condition a program was outlined for a more intensive study and the results are presented in the following pages.

EXPERIMENTAL. A group of albino rats under observation by Samuels in the department of Physiological Chemistry were kept on a basal ration diet consisting of purified casein 20 parts, vitamine B-free starch 64, butter fat 8, cod-liver oil 2, agar agar 4, McCollom salt mixture 4. This diet con-

[1] This investigation has been aided by a grant from the committee on research in problems of sex of the National Research Council; grant administered by F. R. Lillie.

tained neither the heat-stable nor the heat-labile fractions of vitamine B. The larger part of the animals were given this diet ad libitum and also fed 0.5 gram of autoclaved yeast daily. This yeast was air-dried and then autoclaved for four hours at 120°C., thus destroying the heat-labile fraction.

TABLE 1

SERIES	DAYS ON DIET	BODY WEIGHT			CONDITION AT DEATH OF		
		Initial	Final	Per cent loss	Testis	Prostate	Seminal vesicles
A. Vitamine B-deficient diet							
217–397	6	215	191	11	4	4–	4–
218–375	10	197	174	12	4–	4	4–
219–391	10	216	201	7	4–	4	4
233–448	14	225	153	32	4	3–	0
231–442	20	227	167	27	4–	4–	3–
232–395	20	236	173	27	4	4	4–
205–306	40	266	172	35	4	2	0
204–325	54	297	195	34	4	3	1
206–326	61	259	137	47	4–	3–	0
211–361	70	190	126	34	4	3–	0
212–349	70	196	106	46	4–	2	0
210–311	73	256	120	53	4	2	0
B. 50 per cent vitamine B requirement added to diet							
221–434	70	54	138	+155	4–	4–	3
222–435	70	53	135	+155	4–	4–	4–
C. Inanition plus excessive vitamine B requirements							
220–481	24	220	169	23	4	4–	3
228–282	33	210	125	40	3	1	0
229–283	33	231	136	41	4–	1	0
230–242	33	331	222	33	4–	1	0

The condition of the testis, prostate and seminal vesicles is designated by graded ratings. For the prostate and seminal vesicles 4 represents the normal secretory condition and 0 the castrate type, with 3, 2 and 1 representing approaches toward the castrate condition. Testis ratings of 4 indicate normal states, 4– a slightly atypical condition, and 3 mild degeneration of germinal epithelium.

Rats on this diet would lose weight rapidly and develop severe polyneuritic convulsions before death.

Two animals (B, table 1) were given in addition 15 cc. of milk which supplied half of the amount of the heat-labile fraction needed for normal growth. Four animals (C, table 1) were fed 0.5 gram of fresh air-dried yeast daily to supply an excess of both fractions of vitamine B and the intake of the basal ration was limited to progressively decreasing amounts of

8 grams daily for five days, 7 grams for three days, 5 grams for nine days, 3 grams for seven days, thus simulating the loss in weight of the animals on the polyneuritic diet.

At desired intervals the diet-deficient animals were sacrificed and the reproductive systems studied by Moore and Miss Dorothy Price. The study of testes, prostate gland and seminal vesicles provided a means by which effects of the diet could be studied both upon the spermatogenetic and endocrine function of the testis, for it was early apparent that the endocrine production was at fault.

In table 1 an outline summary of findings is presented. The testes of all eighteen animals of this group were essentially normal in gross size and appearance despite the fact that some animals had suffered a weight loss of 40 per cent. These organs for the most part contained histologically normal seminiferous tubules. Some of them possessed tubules containing small quantities of debris from degenerating cells; at times small areas were involved or only separate tubules. By far the majority of tubules were normal and showed not only quantities of mitotic figures but also a normal content of spermatozoa. The majority of them, at death, contained spermatozoa in the epididymis that were proved capable of motility when suspended in normal saline solutions.

The interstitial cells appeared somewhat atypical in that the cytoplasm was greatly reduced in amount and the nuclei were somewhat atypical.

Gametogenetically, therefore, the testes from these diet-deficient animals were essentially normal. Slight amounts of germinal epithelium degeneration are indicated in the table by the designation of 4— where 4 indicates the normal state.

Upon examination, the prostate gland and seminal vesicles were found to vary exceedingly in their approach to the normal state and exhibited a progressive approach towards the castrate type as the length of time upon the deficient diet increased. More specifically these organs tended to exhibit progressive grades of degeneration parallel to the loss of total body weight. Thus animal 233–448 lost 32 per cent of its body weight after fourteen days upon the diet whereas animal 232–395 decreased only 27 per cent in weight after twenty days. The accessory glands of the latter were approximately normal whereas those of the former were more degenerate. When animals attained a weight loss of from 30 per cent to 40 per cent the male accessories were usually found to be quite like those obtained from animals whose testes had been removed some weeks previously.

It became clear from a study of these conditions that the testicles had suffered a loss of their hormone secreting capacities while spermatogenesis progressed normally, or that the accessories were unable to react to hormone if it was being produced.

Two young males (221–434 and 222–435) provided with a diet similar to

the others excepting that 50 per cent of the vitamine B requirements had been added gained much more than 100 per cent in weight and possessed essentially a normal reproductive tract. The testes were all but normal throughout, and the accessories were either normal or slightly below normal in their secretory activities. Both spermatogenesis and the production of internal secretion were progressing in a fashion essentially like that in an animal upon a normal diet.

The question whether absence of B was specifically responsible for the conditions described was investigated by providing inanition diets. Animals were kept with insufficient amounts of the basal ration with excess of dried yeast as a source of vitamine B until they attained a loss in body weight of approximately 33 per cent. A similar loss of weight upon B-deficient diets had usually been accompanied by a castrate type of accessory glands.

Examination of the four animals (C, table 1) made it apparent that similar conditions were present in animals on the inanition diet with excess of B, and in those on unlimited diets lacking in B. In each group the testes were normal in size and the seminiferous tubules were in active spermatogenesis and showed but little or no destruction of germinal epithelium. The prostate gland and seminal vesicles, however, were castrate in type in three of the four animals.

It is to be understood from this, therefore, that a general nutritive deficiency is basically responsible for the peculiar hormone state of these animals rather than any specific effect attributable to absence of vitamine B.

Testis hormone and hypophysis hormone injection. It became of interest to investigate more thoroughly the seminal vesicle and prostate gland condition. Are these structures castrate in type because of the lack of testis hormone necessary to maintain their normal secretory state, or is their condition due to the inability to function because of the low nutritive state of the organism? A separate group of animals was kept upon a vitamine B-deficient diet until they had experienced a loss in body weight of 30 per cent to 40 per cent when it was anticipated that the castrate condition of accessories would have developed. At this time the animals were operated for removal of one seminal vesicle to serve as a record of their hormone state of the moment. They were returned to the B-deficient diet and continued to show a decline in weight until killed. Beginning one day after removal of the seminal vesicle ten animals were given daily injections of testis hormone prepared and standardized by T. F. Gallagher and E. B. Womack in the department of Physiological Chemistry. Approximately six bird units as defined by Gallagher and Koch (22), roughly the equivalent of one rat unit as defined by Moore and Gallagher (23), were injected daily for six to twelve days when the animals were sacrificed for final study.

Four animals were given daily injections of 1 cc. hypophysis hormone, prepared by Prof. H. B. Van Dyke and Z. W. Lawrence in the Pharmacological Laboratories[2] beginning one day after removal of one seminal vesicle and continued for ten days when the animals were sacrificed.

These two series of animals (see table 2), therefore, presented for study seminal vesicle 1 registering the hormone state of the animal at operation, and seminal vesicle 2 for the same purpose after the animal had received injections of testis hormone or hypophysis hormone. Testes and prostate glands were likewise preserved at death and afforded additional material

TABLE 2

ANIMAL	DAYS ON DIET	BODY WEIGHT			SEMINAL VESICLE 1	DAYS OF INJECTION	WEIGHT LOSS AT DEATH	TESTIS	PROSTATE	SEMINAL VESICLE 2
		Initial	At operation	Per cent loss at operation						
A. Testis hormone injection										
							per cent			
280–529	24	179	137	23	0	10	34.5	4−	4	4−
279–492	24	189	136	28	4−	10	44.9	4−	—	4−
247–405	32	226	163	27	0	11	30.5	4−	4	4
248–459	32	218	160	26	0	11	30.9	3	4−	4
249–461	32	229	147	35	0	11	34.1	4−	4−	4
251–427	32	211	135	36	0	11	43.6	3−	3	2
252–425	32	248	169	32	0	10	46.0	4−	4	4−
253–450	32	228	147	35	4	10	37.0	4−	4	4
277–494	36	190	121	34	3−	10	46.5	2	4−	4−
278–372	60	186	128	31	3	10	36.6	3	4−	4−
B. Hypophysis secretion injection										
281–581	24	187	133	28	0	10	36.4	4−	4	4
282–527	24	216	165	23	0	10	34.5	3	4	4
246–457	32	212	135	36	1	10	45.5	4−	4	4
284–548	36	211	152	28	0	10	45.0	4−	4	4

for the study of effects of the injections. The findings for each animal are tabulated in table 2.

For the sake of clarity one animal each from the testis hormone injected group and the hypophysis hormone injected group may be referred to in some detail.

Animal 247–405 was maintained upon the B-deficient diet for 32 days, at which time it had lost 27.9 per cent of its initial weight. At this time

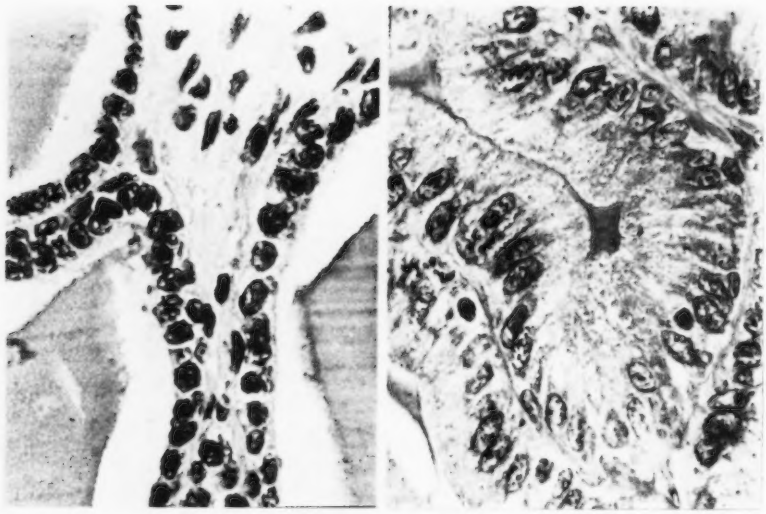
[2] The substance designated hypophysis hormone was prepared from human pregnancy urine and was oestrin free. The method of preparation and standardization will be presented elsewhere.

it was operated for removal of one seminal vesicle which was preserved and studied for determination of the hormone state of the animal. Its condition was rated O, or a castrate type. Within one to two days following operation, testis hormone was injected daily for eleven days in the amount of approximately six bird units, or one rat unit. The animal continued to lose weight during the injection period and when killed it showed a total loss of 39.5 per cent of its initial weight. The testes, prostate gland and remaining seminal vesicle were prepared for study to enable us to determine the reactions of accessories to the hormone injections. The testes proved to be quite normal; 4− indicates that slightly atypical conditions existed. A few seminiferous tubules throughout the testis showed some loss of germinal epithelium, but the majority of them were normal in appearance and contained a full germinal epithelium including normal spermatozoa; mitotic activity was pronounced. The prostate gland was entirely like the normal, hence its rating of 4. The seminal vesicle was likewise in a highly active secretory condition and the cells contained secretion granules. Its normal state, designated by 4, is to be contrasted with the opposite seminal vesicle removed at operation before injections were begun, with a rating of O.

Here, therefore, we have the cytological character of the seminal vesicle to register the hormone condition of the same animal before and after testis hormone injection. Figure 1 pictures the state of the seminal vesicle at operation and figure 2 that of the second seminal vesicle after eleven days of injection. The first one is entirely similar to vesicles removed from animals that had been castrated for some weeks. The secretory epithelium is of a height scarcely greater than the nuclear diameter and no secretion granules are present. The second vesicle, after injection, is entirely similar to those from normal animals; it possesses a high secretory epithelium and secretion granules are abundant. Figure 3 likewise shows that the prostate gland was in a normal secretory state at the time of death. The secretory cells contain the diagnostic light areas which are absent within four days after withdrawal of hormone.

It becomes clear, therefore, that the castrate condition of the accessories before injection was due not to their inability to react to hormone, but because hormone was not being secreted by the intact testes in sufficient amounts to be effective. It is indeed remarkable to realize that whereas the animal as a whole was consistently losing weight the accessory organs responded specifically to testis hormone introduction and doubled or tripled their size within a few days. Their metabolism must have been remarkably affected to permit their sudden increase in an organism consistently on the decline.

Animal 246 457 was treated in an entirely similar fashion to the one described above with the exception that it received injections of hypophysis



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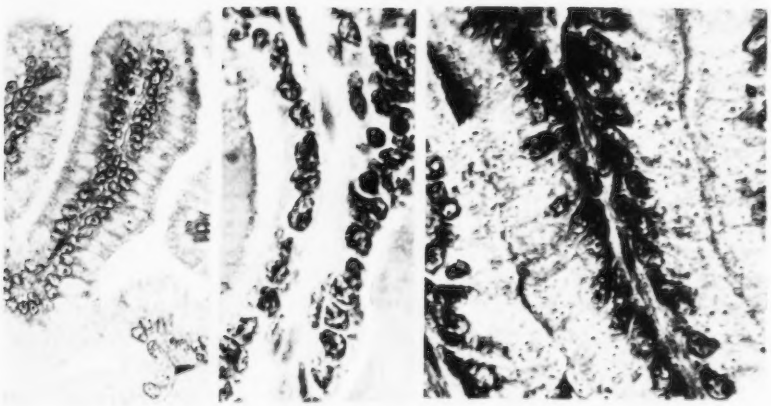

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Fig. 1. Photomicrograph (× 1000) of seminal vesicle 1 from animal 247–405, 32 days on vitamine B-deficient diet.

Fig. 2. Seminal vesicle 2 (same animal as fig. 1) after 11 daily injections of testis hormone (× 1000).

Fig. 3. Prostate gland from same animal as figures 1 and 2 after injections (× 500) (fig. 1 shows castrate type; figs. 2 and 3 are normal).

Fig. 4. Photomicrograph of seminal vesicle 1 (×1000) from animal 246–457, 32 days on vitamine B-deficient diet.

Fig. 5. Seminal vesicle 2 (×1000) from same animal as figure 4 after 10 injections of hypophysis hormone (fig. 4 is castrate type; fig. 5 is normal).

hormone instead of testis hormone. Figure 4 reveals that the operatively removed seminal vesicle was of the castrate type and figure 5 that the second seminal vesicle was entirely normal after ten daily injections of hypophysis hormone. Since the same preparation of hypophysis hormone injected into the castrated male was without effect upon the seminal vesicles it becomes apparent that the action in the animal deficient in the antineuritic factor was indirect. The secretion evidently stimulates the testis to secrete hormone, which it did not formerly do, and the seminal vesicles as well as the prostate gland responded immediately with a return to normal.

By similar procedures we have also determined that animals which had developed the castrate condition in the seminal vesicles while upon the B-deficient diet would return to the normal state if whole yeast were added to supply the deficiency instead of receiving injections of hormone preparations. For example, animal 244–371 was kept on the deficient diet for thirty days and lost 37 per cent of its initial body weight. Whole yeast was added to the diet for a period of eleven days and when killed it registered a total weight loss of only 12 per cent. Examination of the testis, prostate and seminal vesicle showed these structures to be entirely normal.

Animals whose seminal vesicles were reduced to the castrate state by insufficient basal rations but supplied with whole yeast would respond to testis or hypophysis hormone introduction despite the fact that the whole yeast was withdrawn and the diet deficient in B substituted. Thus animal 270–550 was kept on the insufficient basal ration and whole yeast for a period of 25 days, at which time it had experienced a reduction in total body weight of 28 per cent. Seminal vesicle 1 removed at operation was castrate in type. The animal was transferred to the unlimited diet lacking in B for ten days, during which time it received daily hypophyseal hormone injections and the reproductive accessories as well as the testis were found to be entirely normal. The same experiment was repeated using testis hormone for injection instead of the hypophysis hormone and the animals returned similarly to the normal state of the reproductive system.

Discussion. Our chief interest in this study of vitamine B deficiency has centered around the very interesting lack of correlation between the condition of the testis and the accessory reproductive glands. Others (1 to 11) have studied the general effect of B-deficient diets upon reproduction and the reader is referred to these papers for a review of the literature.

Our consistent findings of testes in active spermatogenetic conditions but with castrate types of prostate glands and seminal vesicles raised the question immediately whether the accessories were castrate in type because they were unable to respond to testis hormone or because the testes were for some reason not carrying on their endocrine function. It is now well known that the gametogenetic and endocrine functions of the testis are unrelated.

The injection of testis hormone and the immediate response (within ten days) of the reproductive accessories shows clearly that these accessories are capable of a normal response and function if only the hormone is present. It is furthermore clear that the intact testes are capable of producing or secreting hormone if the substances that stimulate testis function are present. The beautiful work of Smith and Engle (24), Smith (25), and Zondek and Aschheim (26) afford a clear demonstration that gonadal activity is definitely linked with sex-stimulating substances produced by the hypophysis. Our results show that introduction of hypophysis hormone returns the castrate type of accessories to the normal functional type within a period of ten days. This secretion has been found to be without effect upon the accessories of castrated animals; therefore the results obtained by us can only be interpreted as an indirect action. The hypohysial secretion injected into the animal stimulates the testes and they can then secrete their hormone. The testis hormone so produced acts directly upon the accessories, stimulates their metabolic activity and causes them to increase in size and renew their normal functional activity despite the fact that the animal as a whole consistently loses weight.

Having demonstrated that the testis is capable of producing its hormone, provided favorable conditions exist, and that the accessories can respond to the stimulating action of hormone when this is available, the question arises why do the intact testicles not secrete hormone when the animal is maintained upon vitamine B-deficient diets? The study of animals upon inanition diets containing excessive vitamine B requirements demonstrates that absence of B is in no sense specific for absence of testis hormone production.

In another place a general theory has been developed to explain particularly the results obtained from a study of the question of sex hormone antagonism (27) which is likewise applicable to the findings here reported. In brief, it has been assumed that the low nutritive state of the organism affects the hypophysis in such a manner as to minimize the gonadal stimulating substances available to the organism, and upon which the testis depends for its general function. It remains an open question whether rate of production or rate of liberation into the organism of the gonadal stimulating hypophysial substances is the fundamental condition. We must assume also that the gametogenetic function of the testis exhibits a lower threshold than the endocrine function for the latter function ceases while the former continues active.

It is of great interest to note that recently direct evidence for lowered capacity of sex stimulation exists in the hypophysis of such animals as those with which we have been dealing. Evans and Simpson (28) reported before the American Association of Anatomists that hypophyses of animals maintained upon a B-deficient diet were lower in sex stimulating capacity

than those from normal animals, and Mason and Wolfe (29) reported at the same time similar findings regarding the hypophyses from animals upon an inanition diet.

Marrian and Parkes (30) noted that female rats fed vitamine B-free diets, as well as inanition diets, ceased to exhibit oestrous cycles and the follicles in the ovaries were atretic. They, likewise, interpret the defect as a primary hypophysial involvement and restored normal ovarian function and oestrous cycles by subcutaneous injections of macerated hypophysis substance.

It would thus appear that the conditions described by us receive their proper explanations in terms of the more general theory advanced elsewhere and to which reference is given above.

SUMMARY AND CONCLUSIONS

Testes of male rats kept upon a diet deficient in the antineuritic fraction of vitamine B, or on insufficient diets containing an excess of B, were found to consist of normal seminiferous tubules in active spermatogenesis but the reproductive accessories (prostate gland and seminal vesicles) were castrate in type.

Daily injections of either testes hormone, or anterior hypophysis hormone from pregnancy urine, into animals with castrate accessories caused the castrate condition to be replaced by the normal state within a period of ten days.

We interpret these findings in terms of a more general theory advanced elsewhere in the following manner: Partial starvation induced by diets deficient in vitamine B, or insufficient diets rich in B, leads to a poor nutritive state in the organism which affects the hypophysis. The inhibition of hypophysial secretion leads to a lowered amount of the sex stimulating hormone available in the organism. In the absence of this stimulating substance the testes fail to continue their endocrine function and castrate accessories are present. Injections of testis hormone stimulate the accessories directly, whereas injections of hypophysis hormone stimulate the intact testes to secrete their hormone which then acts upon the accessories.

There is evidence to show that under the conditions of the experiment the endocrine function of the testis is disturbed, whereas spermatogenetic function continues.

Acknowledgments. We acknowledge with gratefulness our debt to Prof. F. C. Koch, T. F. Gallagher and E. B. Womack of Physiological Chemistry for active testis hormone preparations, and to Prof. H. B. Van Dyke and Z. W. Lawrence of the Pharmacological Laboratories for active sex stimulating hypophysial hormone. The recovery phases of this problem could not have been thus studied but for the generous coöperation received.

BIBLIOGRAPHY

(1) MASON, K. E. 1925. Proc. Nat. Acad. Sci., xi, 377.
(2) MASON, K. E. 1926. Journ. Exp. Zoöl., xlv, 159.
(3) MASON, K. E. 1929. Journ. Nutrition, i, 311.
(4) MASON, K. E. 1930. Journ. Exp. Zoöl., lv, 101.
(5) MATTILL, H. A. 1927. This Journal, lxxix, 305.
(6) MATTILL, H. A., J. S. CARMAN AND M. M. CLAYTON. 1924. Journ. Biol. Chem., lxi, 729.
(7) PARKES, A. S. AND J. C. DRUMMOND. 1926. Brit. Journ. Exp. Biol., iii, 251.
(8) EVANS, H. M. 1928. Journ. Nutrition, i, 1.
(9) EVANS, H. M. 1928. Journ. Biol. Chem., lxxvii, 651.
(10) EVANS, H. M. AND K. S. BISHOP. 1922. Journ. Met. Res., 1, 335.
(11) EVANS, H. M. AND G. R. BURR. 1927. Memoirs Univ. of California, viii, 1.
(12) MOORE, C. R. 1927. Proc. Soc. Exp. Biol. and Med., xxiv, 847.
(13) MOORE, C. R. 1928. Journ. Exp. Zoöl., l, 455.
(14) MOORE, C. R 1928. Biol. Bull., lv, 339.
(15) MOORE, C. R. AND L. C. MCGEE. 1928. This Journal, lxxxvii, 436.
(16) MOORE, C. R. AND T. F. GALLAGHER. 1929. This Journal, lxxxix, 388.
(17) MOORE, C. R. AND T. F. GALLAGHER. 1930. Amer. Journ. Anat., xlv, 39.
(18) MOORE, C. R., D. PRICE AND T. F. GALLAGHER. 1930. Amer. Journ. Anat., xlv, 71.
(19) MOORE, C. R., W. HUGHES AND T. F. GALLAGHER. 1930. Amer. Journ. Anat., xlv, 109.
(20) MOORE, C. R., S. VATNA AND T. F. GALLAGHER. 1930. Anat. Rec., xlv, 233.
(21) VATNA, S. 1930. Biol. Bull., lviii, 322.
(22) GALLAGHER, T. F. AND F. C. KOCH. 1930. Journ. Pharm. Exp. Therap., xl, 327.
(23) MOORE, C. R. AND T. F. GALLAGHER. 1930. Journ. Pharm. Exp. Therap., xl, 341.
(24) SMITH, P. E. AND E. T. ENGLE. 1927. Amer. Journ. Anat., xl, 159.
(25) SMITH, P. E. 1930. Amer. Journ. Anat., xlv, 205.
(26) ZONDEK, B. AND S. ASCHHEIM. 1927. Klin. Wochenschr., vi, 248.
(27) MOORE, C. R. 1930. Proc. 2nd International Congress for Sex Research (in press).
(28) EVANS, H. M. AND M. E. SIMPSON. 1930. Anat. Rec., xlv, 216.
(29) MASON, K. E. AND J. M. WOLFE. 1930. Anat. Rec., xlv, 232.
(30) MARRIAN, G. F. AND A. S. PARKES. 1929. Proc. Roy. Soc. B., cv, 248.

THE INFLUENCE OF TESTIS HORMONE UPON THE OESTROUS CYCLE IN THE RAT[1]

I. A. IHRKE AND F. D'AMOUR

From the Department of Zoölogy and the Department of Physiological Chemistry and Pharmacology, The University of Chicago

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The preparation of testicular extract of proven potency by Koch and his students (1, 2, 3, 4) has made it possible to study the effects of the testis hormone in the organism in a manner hitherto impossible. Due to the efforts of Moore and his associates (5 to 15) adequate methods for hormone detection in the laboratory mammal have been developed and applied on the male, but up to the present little attention has been given to the introduction of testis hormone into the female.

At the suggestion of Prof. Carl R. Moore and under his direction a study of the influence of hormones introduced into normal and spayed female rats was undertaken and certain of the observations appear to be worthy of record. This paper embodies another of the reports on hormone studies that have materialized from the coöperative study of sex problems between the departments of Zoölogy and Physiological Chemistry and Pharmacology. All materials utilized for injection have been prepared and tested in the latter department, the testis hormone from bull testes by Gallagher and Koch, oestrin from human placentae and pregnancy urine by D'Amour, hypophysis secretion from pregnancy urine by Professors H. B. Van Dyke and Z. W. Lawrence, and extracts of bull brain, heart and liver by D. Edwards under the direction of R. G. Gustavson. We take delight in acknowledging our great indebtedness for materials and guidance during this study.

EXPERIMENTS. A preliminary study of the normal cycle in the rat was made by the vaginal smear method in order to appreciate the limits of normal variation before studying the effects from injection, and all females that failed to show good cycles were discarded.

The colony of females employed proved to be quite typical as the average time duration of 330 cycles recorded within a few weeks was 4.7 days (minimum 2 days—maximum 9 days). All vaginal smears were recorded in

[1] This investigation has been aided by a grant from the committee on research in problems of sex of the National Research Council; grant administered by Professor F. R. Lillie.

terms of the five stages of Long and Evans (16), stage 2 being the cornified oestrous smear and stage 5 the leucocyte or dioestrous resting stage.

A. It is now well known that removal of ovaries brings on the dioestrous smear within two to three days after operation; that one or two injections of oestrin induce the cornified smear (stage 2) within 36 to 48 hours; that the cornified stage remains permanent while daily injections are continued; and that the dioestrous smear again appears and remains permanent when injections cease. These facts have been confirmed by injections of daily doses of twelve rat units of oestrin.

B. A number of ovariotomized females were injected with testis hormone in order to determine whether the dioestrous smear was in any way modified. Daily injections of testis hormone sufficient to substitute for the testis and retain the male accessories in a normal state were used (6 to 10 bird units) (4). Such daily injections into ovariotomized females for a period of two to three weeks were accompanied by the dioestrous smear (stage 5).

The conclusion is justified that testis hormone does not stimulate the female reproductive tract to activity after removal of ovaries.

C. Since the daily injection of oestrin causes the appearance and persistence of the cornified vaginal smear in spayed females it was desired to determine whether addition of testis hormone in any way interfered with the activity of oestrin or the capability of the female tract to respond to oestrin. To this end a number of spayed females were injected daily with mixtures containing 12 rat units of oestrin and 6 to 7 bird units of testis hormone. This amount of oestrin is the same as used above and is sufficient to bring on oestrus in 36 hours, while the amount of testis extract is sufficient to completely substitute for the testis in the male rat. In all injected spayed females the oestrous smear was characteristic as long as injections continued except that very occasionally a leucocyte smear appeared for one day but was replaced immediately by the cornified stage. The same condition was encountered occasionally with oestrin injections alone and hence is considered insignificant.

The findings in such experiments as these are illustrated by one graph (animal 13). It can be seen from this graph that this particular animal gave two leucocyte smears during the 33 days' injection with testis hormone and oestrin mixtures. This animal was chosen for illustration because of the fact that it had received testis hormone injection alone for a period of nine days without modification of the leucocyte smear. It can be seen that within two days after the last mixed hormone injection had been given the animal returned to the dioestrous smear and continued thus until discarded.

The conclusions seem warranted that in mixed potent doses of oestrin and the testis hormone the only effect apparent is the oestrin effect. Chemical contact with testis hormone in no apparent way affected the activity of oestrin and the presence of testis hormone in the female organ-

ism in no way prevented the oestrin from stimulating the oestrous type of vaginal smear. This type of experiment was carried out on fifteen females.

D. Testis hormone was injected into normal females, proved to be running consistent cycles, in order to determine whether its introduction inter-

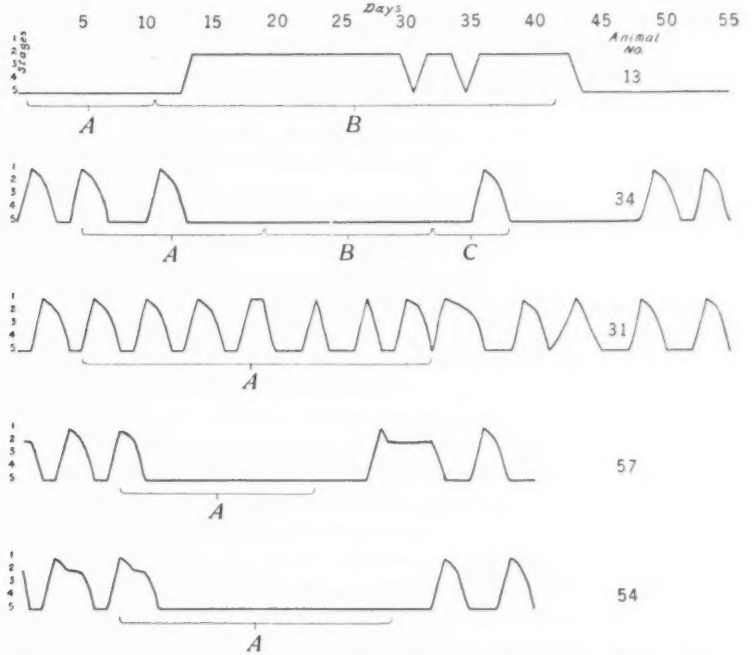

Fig. 1. Graphs recording stages in oestrous cycles of rat. Animal 13. Spayed female running consistent leucocyte or dioestrous smear. A, injected daily with 6 bird units of testis hormone; no effect. B, injected daily with mixtures containing 6 b.u. testis hormone plus 12 rat units oestrin; oestrous smear.

Animal 34. Normal female running normal cycles. A, daily injections of 6 b.u. testis hormone. B, daily injections of 10 b.u. testis hormone. C, daily injections of 10 b.u. testis hormone plus dilute hypophysis hormone.

Animal 31. Normal female running oestrous cycles. A, daily injections with lipoids extracted from bull liver.

Animals 54 and 57. Normal females running cycles. A, daily injection of 9 b.u. testis hormone.

fered with the normal cycles. The experiment was repeated four or five times with five different samples of testis hormone and it was found that introduction of testis hormone caused a suppression of the typical cycle. It was noted early that with amounts of testis hormone at or slightly under an amount necessary to show complete testis replacements in the male, the

cycles became quite irregular or were entirely suppressed. When the dosage was increased by one-half, however, complete suppression of the cycle was noted.

This result may be illustrated by reference to one group of fifteen females. The group was carefully selected from twenty-five females under observation for cyclic changes and only those running consistent cycles over a period of thirty days were utilized. Injections of six bird units of testis hormone (approximately one rat unit as defined by Moore and Gallagher, 18) were given daily for twelve days. Eleven of the females showed within this period only an occasional cornified smear, whereas smears from the other four were consistently of the dioestrous leucocyte type. The dosage for all was increased by one-half and continued for twelve days. During this period none of the fifteen females gave a cornified smear. Six of the females were continued on the daily testis hormone dose but were given in addition 1 cc. of a diluted hypophysis secretion for five days. Four of the six gave a cornified smear within two days after hypophysis secretion injection despite the fact that they received also the testis hormone doses that had previously suppressed the cycle. All animals returned to the normal consistent cycle shortly after discontinuing all injection. This type of result is shown graphically for animal 34 in figure 1.

As a control against the injection of testis hormone six females were injected daily with lipoid extracts of bull heart, brain or liver for a period of twenty-five days. The cycles in all these females continued with regularity throughout the injection period and showed no effect from the lipoids of other tissues. The graph of animal 31 pictures the results with an animal receiving daily injections of lipoid extracts from bull liver.

Another small group of females illustrated again the parallelism between testis hormone injections and cycle suppression. From a group of eight females running consistent cycles four were selected for injection and four remained as controls. Each of the four received a daily injection of nine bird units of testis hormone—two for a period of 17 days and two for 24 days. One animal of the four showed two cornified smears within the first five days of injection, whereas the other three gave only leucocyte smears. Both of those injected for 17 days gave a typical cornified smear on the fifth day after the last injection and both of those injected for 24 days gave the oestrous smear on the fourth day after the last injection. All four uninjected controls gave consistent smears throughout the period. Animals 57 and 54 (see graph) show this result for the 17 and 24 day period.

It is therefore apparent that injection of testis hormone effectively suppresses the female oestrous cycle during the period of injection and that when injections cease the normal cycle is resumed. The length of cycle suppression is parallel to the injection period. Furthermore, while testis hormone injection is continued the oestrous smear may be induced by introduction of hypophysis secretion.

Discussion. The question of the influence of the hormones when present at the same time in the same organism has received a great deal of attention since Steinach about 1910 postulated an antagonistic action between the male and female gonads and which Lipschutz and Steinach more recently have applied to the hormones themselves.

The results obtained from introducing oestrin and testis hormone into the spayed and normal females, singly and together as mixtures, may be briefly expressed as follows:

A. Testis hormone does not change the vaginal smear in the spayed female. B. Oestrin brings forth a cornified vaginal smear in spayed females within 36 hours. C. Oestrin, when mixed with testis hormone, is apparently unmodified in its activity. The mixtures induce the oestrous smear as readily as when oestrin alone is introduced. There is no apparent chemical neutralization of oestrin by testis hormone and the presence of the latter did not modify the capacity of the female tract to respond to oestrin. D. Testis hormone introduced into normal females brings forth the dioestrous smear and effectively obliterates cycles so far as these are indicated by the vaginal smear technique; the dioestrous smear remains permanent as long as injections are continued and when injections are discontinued the typical cycle is resumed. E. During the period of testis hormone injection, and the suppressed cycle in normal females, the introduction of hypophysis secretion induces the cornified smear.

The question of the interpretation of these results is before us. Results obtained during the past three years by Moore and his associates including observations made upon castrated and normal males 1, injected with testis hormone and oestrin—singly or mixed—2, with fresh anterior lobe hypophysis implants in various combinations with hormone injection; 3, with hypophysectomies and hormone injection; 4, under conditions induced through partial starvation along with injections, and 5, the results herein reported upon the female, led to the formulation of a conception of hormone action within the organism that likewise offers a logical interpretation for the present results (17).

The conception is based fundamentally upon certain ideas, some of which have received definite proof, and which may be briefly stated as follows: 1. Sex hormones, whether produced by intact gonads or introduced by injection, stimulate the homologous sex characters in the organism but do not influence heterologous characters. 2. Sex hormones do not have a direct influence upon either the homologous or heterologous gonad. 3. The sex stimulating secretion of the hypophysis controls the two functions of the gonad—the gametogenetic and endocrine functions. Without the presence of this secretion both gonad functions are in abeyance. 4. Gonad hormones of either sex act to depress the hypophysis and result in a lessened amount of hypophysis sex stimulating secretion available to the organism.

These fundamental ideas have been applied by Moore to explain a large series of observations, made particularly upon the male, but applied generally to explain certain phases of female activity.

Applying the general conceptions to the results here reported we find A, that testis hormone does not influence the vaginal smear in spayed females since it does not act upon heterologous characters—here the female reproductive tract. B. Oestrin stimulates the reproductive tract of spayed females and brings on the oestrous smear, hence stimulating the homologous characters. C. Mixtures of oestrin and testis hormone into spayed females give results attributable to oestrin alone. The testis hormone is without effect in this case either upon oestrin or the female tract. D. Testis hormone injected into normal females causes suppression of oestrous cycles, not from an action upon the female accessories or upon the ovary, but by acting upon the hypophysis of the female and suppressing its function to the extent that an insufficient amount of sex stimulating hypophyseal secretion is present to permit the intact ovary to produce its secretion. When the ovarian secretion is absent or low the oestrous cycle is irregular or absent. E. Upon such a condition as is brought about under D, the introduction of hypophysis sex stimulating secretions by injection supplies a sufficient stimulant for the ovary to function and a cornified smear again appears.

Such an explanation as here offered follows logically from that offered by Moore to account for a varied group of findings. Indeed the results here reported enter into the conception as presented and form one line of evidence upon which the new interpretation was based. For details of other types of evidence the reader is referred to the general paper and to other papers now in press.

SUMMARY AND CONCLUSIONS

1. Daily injection of testis hormone into spayed female rats does not stimulate the cornified vaginal smear of oestrus or have any demonstrable effect.

2. Injections of mixtures of testis hormone and oestrin into spayed females is accompanied by the cornified cell smear as long as injections are continued. When injections are stopped the leucocyte (or dioestrous) smear appears and is permanent.

3. Testis hormone injected daily into normal females suppresses the oestrous cycle for the period of the injection. When injections are no longer given the normal cycle expressed by the vaginal smear reappears.

4. Normal female rats with cycles suppressed by testis hormone give cornified smears within two to three days after injection of the sex stimulating anterior lobe secretion prepared from pregnancy urine.

5. These findings are interpreted in the light of a broad general conception advanced by others.

BIBLIOGRAPHY

(1) McGee, L. C. 1927. Proc. Inst. Med. (Chicago).
(2) Gallagher, T. F. 1928. This Journal, lxxxvii, 447.
(3) Gallagher, T. F. and F. C. Koch. 1929. Journ. Biol. Chem., lxxxiv 495.
(4) Gallagher, T. F. and F. C. Koch. 1930. Journ. Pharm. Exp. Therap., xl, 327.
(5) Moore, C. R. 1927. Proc. Soc. Exp. Biol. and Med., xxiv, 847.
(6) Moore, C. R. 1928. Journ. Exp. Zoöl., l, 455.
(7) Moore, C. R. 1928. Biol. Bull., lv, 339.
(8) Moore, C. R. and L. C. McGee. 1928. This Journal, lxxxvii, 436.
(9) Moore, C. R. and T. F. Gallagher. 1929. This Journal, lxxxix, 388.
(10) Moore, C. R., T. F. Gallagher and F. C. Koch. 1929. Endocrinol., xiii, 367.
(11) Moore, C. R. and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 39.
(12) Moore, C. R., D. Price and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 71.
(13) Moore, C. R., W. Hughes and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 109.
(14) Vatna, S. 1930. Biol. Bull., lviii, 322.
(15) Heller, R. E. 1930. Proc. Soc. Exp. Biol. and Med., xxvii, 751.
(16) Long, J. A. and H. M. Evans. 1922. Memoirs Univ. of California, vi.
(17) Moore, C. R. 1930. Proc. 2nd International Congress for Sex Research (in press).
(18) Moore, C. R. and T. F. Gallagher. 1930. Journ. Pharm. Exp. Therap., xl, 341.

MUSCLE COUNTS OF MOTOR UNITS: A STUDY IN INNERVATION RATIOS

D. A. CLARK

From the Laboratory of Physiology, Oxford

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The ultimate functional element on the motor side of the reflex arc is designated the "motor unit." This term, which was introduced by Sherrington, was defined by him as follows (13): "The muscle and its nerve may be thought of as an additive assembly of 'motor units,' meaning by 'motor unit' an individual motor nerve fibre together with the bunch of muscle fibres it innervates." The study of such units has been an incidental part of much of the work upon the "all or none" principle. Eccles and Sherrington (5) have lately made quantitative determinations of the isometric contraction tensions developed by individual motor units. They employed the simple expedient of measuring in "deganglionated muscle" the total tension developed during an isometric tetanus and dividing the value so obtained by the number of motor fibres found subsequently in the muscle's nerve. The somewhat surprising information was obtained that a single nerve fibre is capable of producing 10 grams, 20 grams, and even as much as 40 grams contraction tension. In the course of their investigations, the question arose as to how many muscle fibres were included in a single unit and this proved to be the starting point of the present study.

Interest in the number of muscle fibres innervated by a single nerve fibre is by no means new, the problem having been approached in the past in two ways, viz: by tracing down an individual nerve fibre and observing the number of branches into which it divides, and by counting the fibres in a muscle and comparing this figure with the number of fibres in its nerve. The technical difficulties involved in following a single nerve fibre are enormous and although Kölliker (7) Mays (9), Woollard (15), Cajal (1), Cooper (2), Eccles and Sherrington (5) and many others have shown that both sensory and motor nerve fibres divide into several branches, none of the figures obtained has furnished a dependable estimate of the size of a motor unit. On the other hand, several attempts have been made to investigate the question by means of counting the nerve fibres and the muscle fibres belonging to a given muscle. The present study falls within this class, and the ratio of the number of motor nerve fibres supplying a given muscle to the number of its muscle fibres has been here

designated the "innervation ratio." Reichert (12) in 1851 found a ratio of one nerve fibre to 20–30 muscle fibres in M. sterno-cutaneus of the frog. More interesting is the work of Tergast (14) who in 1873 when a candidate for his medical degree at Göttingen, carefully examined the relation of nerve fibres to muscle fibres in a series of different muscles. He found one nerve fibre to about three muscle fibres in the eye muscles of the sheep, but 1:80 and 1:125 in the limb muscles of the dog. Other innervation ratios have been mentioned by Lucas (8), Porter and Hart (11), Fulton (6) and Porter (10). Although some of the ratios given are suggestive, all the calculations were based wholly on estimates. The afferent fibres were allowed for in several cases, but I have found no record of their being experimentally removed; moreover, there is a wide difference of opinion regarding the exact innervation ratio in different animals. For these reasons, it has been deemed worth while to attempt its determination by a more accurate method. The cat was selected for study on account of its widespread use for physiological experimentation. M. soleus was chosen as representative of "red"[1] muscle, while M. extensor longus digitorum was selected as typical of "white" muscle.

Method. Determination of an innervation ratio requires accurate enumeration both of the motor nerve fibres and of the muscle fibres belonging to a given muscle. The methods employed in making these enumerations were as follows:

Enumeration of motor nerve fibres. The afferent fibres in the nerves to M. soleus and M. extensor longus digitorum were excluded by operative removal of the appropriate posterior root ganglia and by allowing a period of 42 to 60 days for complete degeneration of all sensory fibres. Five such operations were performed by Professor Sherrington who at the end of the degeneration period determined the tension developed in a maximal tetanus (torsion wire myograph) of the two chosen muscles. The muscles together with their nerves were then dissected out, and the muscles weighed in "normal" saline solution. The motor fibres remaining in the nerves were then counted by Professor Sherrington using the technique recently described by him (5). Subsequently the removal of all the ganglion cells was controlled by examination of the operated spinal roots in microscopical sections.

Enumeration of muscle fibres. To ensure the greatest accuracy, it was decided to count every individual fibre in each muscle. The simplest

[1] The terms "red" and "white" muscle have been used throughout the paper to designate the two chief kinds of striped muscle. The words are placed in quotation marks, however, on account of the demonstration of Denny-Brown (4) that "redness" and "whiteness" are not necessarily associated with any particular type of function. As used here, the words refer to the classes which he has distinguished as "slow" and "rapid" muscle respectively.

procedure is to do this in cross-sections of the muscles, but the accuracy of the enumerations then depends upon whether or not a cross-section actually includes all the muscle's fibres, and this in turn depends upon the structure of the particular muscle in question. The structure of M. soleus in the cat and of striated muscle in general has been described by Denny-Brown (4). It consists of two aponeuroses (the one of origin and the other of insertion) between which run numerous fasciculi or bundles of muscle fibres. All the fasciculi are parallel, and every one runs the full distance from one aponeurosis to the other. Furthermore, every fibre in a particular fasciculus runs from end to end of the fasciculus. These observations were made in fresh preparations examined under the dissecting microscope and in muscles macerated in 20 per cent nitric acid. They have been repeated in the course of the present work and confirmed in every particular. Any cross-section of the whole muscle will therefore include all of its fibres. This arrangement is illustrated diagrammatically in figure 1 C. In the case of M. extensor longus digitorum this structure is somewhat modified. The muscle consists, as does M. soleus, of two aponeuroses between which the fasciculi run; all the fasciculi are parallel and all run the full distance from one aponeurosis to the other. A certain proportion of the individual fibres, however, do not run from end to end of a fasciculus but taper off in the body of the muscle. This structure is shown diagrammatically in figure 1 D. Measurements of the lengths, after maceration, of ten fasciculi and of twenty individual fibres picked at random from two different muscles show that the average length of a fasciculus is 16.4 mm. while that of a "short" tapering fibre is 11.5 mm. These figures indicate that the tapering ends of the "short" fibres lap over a considerable amount in the body of the muscle, so that any cross-section through the central part of M. extensor longus digitorum will include all of its fibres. As in the case of M. soleus, each individual fibre of the extensor, whether of the shorter or longer variety, gives its silken termination directly to the tendon aponeurosis and by virtue of this beautiful structural arrangement each fibre contributes its share of tension directly to the tendon.

For the purpose of enumeration it is important that every individual muscle fibre be cut at right angles to its long axis, but a glance at figure 1A will show that this is impossible in any single cross-section of the whole muscle, on account of the oblique course taken by the fibres. The muscles were therefore split into three parts with a cataract knife under the dissecting microscope, all the separations being made between fasciculi as at *a* and *b* in figure 1 A, very little injury being done to the fibres. Cross-sections could then be taken of each of the three parts separately as at *x*, *y*, and *z* in figure 1 B with each fibre cut at right angles to its long axis. Every fibre in the muscle was thus included in one of the three cross-sections and no fibre appeared twice.

After the myograph tests had been made and the muscles had been dissected out and weighed (with their tendons removed), they were then split as described above and placed in "Susa" fixative for about 12 hours, pinned out at a considerable stretch in order to keep all the fibres parallel during the process of fixation. They were then embedded in celloidin, and sections of 15μ to 30μ were taken, with care in the case of M. extensor longus digitorum that the sections should be from as near the centre of the

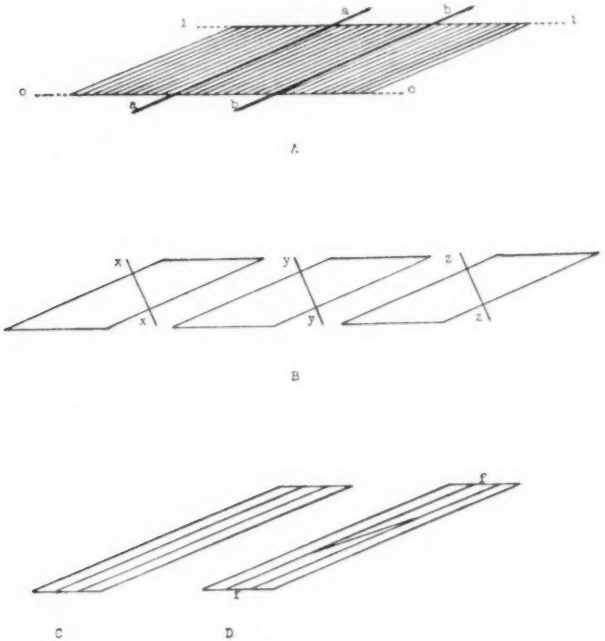

Fig. 1. A. The full muscle. a, b. Points at which the muscle is split. o. Aponeurosis of origin. i. Aponeurosis of insertion. B. The muscle after splitting. x, y, z. Points at which cross-sections are taken. C. Details of a fasciculus of M. soleus. D. Details of a fasciculus of M. extensor longus digitorum. f. "Short" tapering fibres.

muscle as possible. The sections were stained either with Delafield's hematoxylin and with eosin or with Heidenhain's iron hematoxylin alone.

In order to count the muscle fibres in the sections thus obtained the latter were, at the suggestion of Mr. Watson Baker of W. Watson and Sons, Limited, projected on bromide paper at a magnification of 75 to 100 diameters by means of a microprojector. Photographically the projected image is a negative, but for purposes of counting this is of no importance.

The fibres in each photograph were counted by marking each with a dot of white ink, at the same instant tapping an automatic recording device with the foot. Marking with the white ink eliminated the danger of counting any fibre more than once, while the recording device ensured that no fibre would be omitted from the final enumeration. The counting was constantly controlled by reference to the original slide from which the photograph had been made. A method similar to this has been used by Dale (3) for counting nerve fibres. The accuracy of the method was tested by counting the fibres in one section at two separate times three months apart, and an error of only plus or minus 2.6 per cent was found—which was considered to be within experimental limits. The enumeration of each

TABLE 1

NUMBER OF EXPERIMENT	MUSCLE	WEIGHT OF MUSCLE	TOTAL TENSION	NUMBER OF MOTOR NERVE FIBRES	NUMBER OF MUSCLE FIBRES	WEIGHT PER MUSCLE FIBRE	TENSION PER MUSCLE FIBRE	TENSION PER NERVE FIBRE	INNERVATION RATIO
		grams	*grams*			*mgm.*	*mgm.*	*grams*	
1	soleus	3.06	1,940	198	22,012	0.139	90	9.8	1:111
2	soleus	2.63	1,780	174	22,622	0.116	80	10.2	1:130
3	soleus	4.11	560	192	30,351	0.135	20*	2.8*	1:158*
4	soleus	3.04	1,072	187	30,085	0.101	36*	5.7*	1:161*
5	soleus	3.25	2,230	233	27,261	0.119	82	9.6	1:117
6	ext. l. d.	3.95	2,950	315	—	—	—	9.4	—
7	ext. l. d.	2.05	1,450	277	32,396	0.063	45	5.2*	1:117*
8	ext. l. d.	3.31	2,500	297	—	—	—	8.4	—
9	ext. l. d.	2.93	—	244	41,577	0.070	—	—	1:170
10	ext. l. d.	3.16	2,010	247	38,733	0.082	52	8.1	1:157

* Excluded from averages.

muscle was completed before learning the number of its motor fibres, in order to avoid possible prejudiced or "suggested" counting.

RESULTS. The results are summarized in table 1. With certain exceptions, there are four series of observations, and four series of calculations from each of the muscles studied. The four series of observations are: the weight of the muscle (tendon removed); the tension developed at maximal tetanus; the number of motor nerve fibres; and the number of muscle fibres. With these at hand it was a simple matter to make the calculations: dividing the weight of the muscle and the total tension it developed by the number of its muscle fibres gave values for the weight of a single muscle fibre and for the tension developed by it; dividing the total tension developed by the number of motor nerve fibres gave the tension per nerve fibre (or per motor unit); and dividing the number of muscle fibres by the number of motor nerve fibres gave the number of muscle fibres in a single motor unit (the innervation ratio).

Before averages could be taken of the values found in the five individual muscles of each type examined, certain unreliable values had to be excluded. These have been marked with an asterisk in table 1. In the case of experiments 3 and 4 all the calculations which involved either the number of motor nerve fibres or the tension developed by the muscle have been eliminated, because in these animals it was found that a great many motor fibres had been injured in the operations for the removal of the posterior root ganglia, and because the myograph was working badly during the determination of the tensions. In experiment 7, the calculations involving the number of motor nerve fibres have been excluded since microscopical examination of the posterior roots showed that a large number of ganglion cells had not been removed in the operation, so that afferent fibres were present in the nerves when the fibres were counted. In experiment 9, the number of motor fibres (244) was not observed but was calculated on the basis of the number of motor fibres found per gram weight

TABLE 2

MUSCLE	WEIGHT PER MUSCLE FIBRE	TENSION PER MUSCLE FIBRE	TENSION PER NERVE FIBRE	INNERVATION RATIO
	mgm.	*mgm.*	*grams*	
M. soleus	0.122	84.0	9.9	1:120
M. extensor longus digitorum	0.072	48.5	8.6	1:165

of muscle in experiments 6, 8, and 10. It is not considered to be more accurate than plus or minus 5 per cent.

After consideration of these factors, averages of the reliable values were taken and are given in table 2. It must be pointed out that these averages are not regarded as more significant than any of component calculations from which they are drawn up. Comparison of the averages in table 2 with the calculations in table 1 shows that none of the averages is far distant from any one of its components, however. The averages are therefore considered to represent truthfully the relationships concerned, and they will be used as a basis for discussion.

It will be seen that the "red" fibre of M. soleus weighs 0.122 mgm. while the "white" fibre of M. extensor longus digitorum weighs only 0.072 mgm. This variation might be explained by differences in length and diameter between the two types of fibre, and by the presence of the short fibres mentioned above in M. extensor longus digitorum. Such differences, however, as far as can be determined from the information now available, do not appear to be great enough to explain such a large difference in weight, and it is conceivable that the density of the contractile substance of "red" fibre is greater than that of "white," but adequate

data to prove this point are not yet available. The "white" fibre of M. extensor longus digitorum develops an average tension of 48.5 mgm. while the "red" fibre of M. soleus develops 84 mgm., and therefore would seem to be much the stronger.[2] It cannot be shown definitely from the present data, however, whether this greater strength is due to a larger volume of contractile substance per "red" fibre or to an intrinsically greater power of the "red" fibre than that of the "white" fibre of the same size. But again the volume differences between the two kinds of fibre do not appear to be great enough to account for such a large variation in tension developed and it seems likely that the "red" muscle substance is more powerful than the "white," though not so rapid.

The tension developed per motor unit by M. soleus is 9.9 grams and by M. extensor longus digitorum, 8.6 grams. These figures are not far apart from each other and correspond closely to those found for the same muscles by Eccles and Sherrington (5). Since there is a full discussion of the significance of the tension developed per nerve fibre in the paper of these investigators, this question will not be entered into here.

The innervation ratios obtained for the two muscles are interesting and appear to be reliable within plus or minus about 10 per cent. In M. soleus the average motor unit contains 120 muscle fibres and in M. extensor longus digitorum it contains 165 muscle fibres. Both these figures are high and might seem to indicate that delicate fractionation of movement would be difficult for these two muscles. It must be remembered, however, that the figures given represent a mean value only for the number of muscle fibres per motor unit, and that it is probable that individual motor units within a single muscle vary considerably in the amount of tension they develop and in the number of muscle fibres they contain.

The question immediately arises as to how far such high ratios are applicable to striped muscle in general. In the case of any particular muscle, it seems likely that the innervation ratio bears a definite relationship to the function performed by that muscle. For a muscle is strictly divided into only so many functional units as it has motor fibres, and its ultimate capacity for fractional response is determined by this number. On the other hand the number of muscle fibres subserved by a single motor fibre fixes the strength of the unit. Cooper (2) has recently demonstrated the significant fact that in the cat's sartorius the fibres constituting a motor unit do not lie "in parallel" at one end of the muscle, and she points out that such a condition would in fact cause during a fractional response a

[2] This differs from the conclusions of Denny-Brown (4) who finds that the "white" fibre of M. gastrocnemius (median head) is about twice as strong as the "red" fibre of M. soleus in the cat. The cause of this discrepancy is not clear, but it may be due to the very large number of muscle fibres probably contained in the motor unit of M. gastrocnemius.

loss of effectiveness to the muscle as a whole. The fibres lie rather in a chain, she has demonstrated, which extends from end to end of the muscle along its axis. According to the "all-or-none" principle each muscle fibre when stimulated by its nerve must contract to its full capacity or not at all, and the size of the contraction remains constant so long as the conditions of activity are the same. It is then clear that when such a chain, or motor unit, is activated it will always develop, in a given contraction, the highest tension of which it is capable. Fractionation consists therefore in the activation of a greater or smaller number of such units. The central nervous system is consequently limited in its ability to command fractionation of activity by the size and strength of the motor unit in a given muscle. As a result of this, muscles performing functions which demand highly differentiated movements will be expected to have smaller motor units, or lower innervation ratios, than those serving a more ordinary purpose. For example, one may suppose that the interossei and lumbricales have lower ratios than the muscles controlling movements at the shoulder joint, though evidence on this point is not yet available. The lowest ratios might be expected in the muscles of the eye and ear. Unfortunately, there is no method known at present whereby the afferents to these muscles can be removed. Moreover, they cannot be allowed for by calculation, since, though they are very numerous, there is not the scantiest evidence of what their proportion is. Also, as Professor Sherrington has pointed out to me, the nerve fibres to the eye muscles divide very profusely for some distance away from the muscles, which would make accurate enumeration of them difficult, if not impossible.

The significance of the fact that M. extensor longus digitorum has a higher ratio (1:165) than M. soleus (1:120) is not entirely clear. It is possible that for rapid, well synchronized movement, such as that demanded of this extensor, a high ratio is useful; whilst for the slower postural function of M. soleus a smaller one is of greater benefit. There is not, it will be noted, a great difference between them, and a ratio of a completely different order would be expected in muscles capable of finer adjustment. In the case of the median head of M. gastrocnemius in the cat an even higher ratio than those found in this study is almost certainly present. Eccles and Sherrington (5) found that this muscle develops a tension of about 30 grams per motor unit, which is about three times as great as that found in M. extensor longus digitorum. It is unlikely that the individual "white" fibre of M. gastrocnemius is three times as strong as the "white" fibre of M. extensor longus digitorum, especially since the gastrocnemius fibre is much shorter than that of the extensor, so that we must expect an innervation ratio in M. gastrocnemius of 1:300 or 1:400.

With ratios of this order, it must be stressed that a mean value only has been obtained, and that smaller units are also present within the

muscle, and that it is likely that it is these smaller units which are called upon to make the finer adjustments of movement and posture of which even the largest limb muscles are capable.

SUMMARY

1. A method has been described for counting the total number of fibres in skeletal muscles.

2. The motor nerve fibres and muscle fibres have been counted in five examples of M. soleus and in three examples of M. extensor longus digitorum of the cat.

3. The fibre of "red" muscle has been found stronger and of greater density than the fibre of "white" muscle.

4. "Innervation ratio" has been defined as the ratio of the number of motor nerve fibres supplying a given muscle to the number of its muscle fibres.

5. An innervation ratio of about 1 to 120 has been found for M. soleus, and one of about 1 to 165 for M. extensor longus digitorum.

I wish to express my thanks to Sir Charles Sherrington for his helpful advice and encouragement throughout the work, to Dr. H. M. Carleton for the use of his laboratory, and to Dr. J. F. Fulton for his suggestion of the problem. I should also like to thank the Christopher Welch Trustees for a grant to defray the expenses of the investigation.

BIBLIOGRAPHY

(1) Cajal, S. Ramon. 1909, 1911. "Histologie du Système nerveux de l'homme et des vertébrés." 2 tomes. Paris: Maloine. Tome 1, 285.
(2) Cooper, S. 1929. Journ. Physiol., lxvii, 1.
(3) Dale, H. H. 1900. Journ. Physiol., xxv, 196.
(4) Denny-Brown, D. 1929. Proc. Roy. Soc., civ B, 371.
(5) Eccles, J. C. and C. S. Sherrington. 1930. Proc. Roy. Soc. cvi B, 326.
(6) Fulton, J. F. 1926. Muscular contraction and the reflex control of movement. London: Balliere, Tyndall, & Cox. xv + 644 pp. 369.
(7) von Kölliker, A. 1892. Biol. Centralbl., xii, 33.
(8) Lucas, K. 1905, 1909. Journ. Physiol., xxxiii, 125; xxxviii, 113.
(9) Mays, K. 1886. Zeitschr. f. Biol., xxii, 354.
(10) Porter, E. L. 1929. This Journal, xci, 345.
(11) Porter, E. L. and V. W. Hart. 1923. This Journal, lxvi, 391.
(12) Reichert, K. E. 1851. Arch. f. Anat., Physiol., u. wissenschaftl. Med., 29.
(13) Sherrington, C. S. 1925; 1929. Proc. Roy. Soc., xcvii B, 519; cv B, 332.
(14) Tergast, P. 1873. Arch. f. Mikros. Anat., ix, 36.
(15) Woollard, H. H. 1926. Heart, xiii, 319.

COAGULATION TIME OF THE BLOOD OF ADRENALECTOMIZED RATS

L. B. NICE, O. C. IRWIN AND RUTH M. KRAFT

From the Department of Physiology, Ohio State University

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The rate of coagulation of the blood of adrenalectomized rats in the quiet and excited state (anger and pain) was determined in this study. For comparative purposes similar determinatons were made on the blood of normal rats under the same conditions.

Cannon and Mendenhall (1914; also see Cannon, 1929) reported a decided decrease in the clotting time of cat's blood after stimulation of the peripheral end of a transected splanchnic nerve, but none after removal of the adrenal gland on that side. Barlow and Ellis (1924) reported the coagulation time of double adrenalectomized cats as being shorter than before the operation.

METHODS. Our rats had both adrenal glands removed at one operation. The coagulation determinations were made within 2 to 12 days after removal of the glands. This procedure was decided upon as adrenal rests may develop in some of the rats causing them to behave as normal animals.

The apparatus used for measuring the rate of coagulation was a modification of the coagulometer devised by Cannon and Mendenhall (1914). The animals were lightly anesthetized with ether and the blood quickly drawn from the heart by means of a 2 cc. Luer hypodermic syringe having a one-half inch no. 26 needle. As soon as the blood appeared in the syringe a stop watch was started running. The blood was quickly transferred into a 2 mm. glass tube approximately 2 cm. long, sealed at the lower end, placed upright in a water bath having the temperature maintained at 25°C. The time when the clot supported the plunger was taken as the end point for the clotting.

After having drawn the blood in the quiet state the rats were permitted to recover from the ether. They were then teased for five minutes by being stimulated from time to time with a medium strength faradic current. Blood was again quickly drawn as above and the clotting time determined. The second test was made approximately 25 minutes after the first.

RESULTS. In the normal rats there was a marked decrease in the coagulation time after excitement. The average for 15 rats in the quiet state as shown in table 1 was 3 minutes, 23 seconds, and after stimulation,

TABLE 1

Clotting time in normal rats

SEX	BEFORE STIMULATION		AFTER STIMULATION	
	minutes	*seconds*	*minutes*	*seconds*
Female	4	25	1	45
			1	23
Male	2	50	2	29
			1	50
Male	3	21	1	19
Male	4	0	1	8
Male	3	0	2	40
Male	3	0	1	20
Male	3	45	1	27
Female	2	15	1	50
Male	4	16	2	0
Male	3	3	2	15
			1	45
Male	3	40	1	55
Male	3	58	1	55
Male	2	53	1	45
Male	4	25	1	51
Male	2	2	1	32
Average.....	3	23	1	47

TABLE 2

Clotting time in adrenalectomized rats

SEX	NUMBER OF DAYS AFTER OPERATION	BEFORE EXCITEMENT		AFTER EXCITEMENT	
		minutes	*seconds*	*minutes*	*seconds*
Male	2	3	30	2	3
Male	2	2		2	
Male	3	3	45	2	
Female	2	2	52	3	24
				3	30
Female	12	3	25	1	55
Female	6	3	27	3	8
Male	6	3	11	2	12
Male	5	4	55	4	25
Male	3	2	37	3	57
Male	3	3	12	2	11
Male	3	3	9	3	23
Male	5	2	17	1	33
Male	6	3	28	2	17
Male	7	2	50	2	31
Male	2	4	3	3	42
Male	2	2	48	2	16
Male	2	3	32	2	36
Male	5	2	46	2	26
Male	5	2	1	1	48
Average.....		3	4	2	40

1 minute 47 seconds. In every case there was a decided shortening of the coagulation time. Data on other normal animals gave very similar results; 28 determinations on 17 normal rats in the quiet state averaged 3 minutes 14 seconds, while 15 determinations on another group of 10 excited rats averaged 1 minute 44 seconds. In the two groups the average decrease was 47 and 46 per cent respectively.

The adrenalectomized rats showed much the same coagulation time before excitement as the normal animals, but after stimulation the shortening was seldom as great. The average clotting time for 19 adrenalectomized rats before stimulation as shown in table 2 was 3 minutes 4 seconds and afterwards 2 minutes 40 seconds. In 15 determinations there was a decrease, in 5 an increase while in one they were equal. The average decrease for the group as a whole was 13 per cent.

Discussion. We found it impracticable to secure blood from these rodents without an anesthetic. The ether may have influenced the determinations to some extent. The fact, however, that our results on the whole are consistent leads us to believe that for comparative purposes they are valid.

There was a decided shortening of the clotting time of the blood in the normal group after stimulation.

The adrenalectomized rats were less excitable under stimuli than the normal animals and the changes in the rate of coagulation less marked. This lessened excitability accompanied by a decrease in physical exertion may in some degree account for the less marked shortening of the coagulation time in this group (Hartman, 1927).

SUMMARY

1. Our normal rats showed a well defined shortening in the rate of coagulation of their blood (averaging 47 per cent) after being restrained and stimulated.

2. The clotting time of the blood of the adrenalectomized rats after excitement was decreased in 15 determinations, increased in 5, while in one case the clotting time before and after stimulation was the same. The average decrease for the group as a whole was 13 per cent.

3. Our results indicate that the adrenal glands are a large factor, but not the only one responsible for the shortening of the coagulation time of the blood of emotionally excited rats.

BIBLIOGRAPHY

Cannon, W. B. and W. L. Mendenhall. 1914. This Journal, xxxiv, 255.

Cannon, W. B. 1929. Bodily changes in pain, hunger, fear and rage. New York, p. 157.

Barlow, O. W. and M. M. Ellis. 1924. This Journal, lxx, 58.

Hartman, F. A. 1927. This Journal, lxxx, 716.

THE EFFECT OF REGIONAL SYMPATHECTOMY UPON MUSCLE GLYCOGEN

S. DWORKIN,[1] Z. M. BACQ[2] AND D. B. DILL

From the Laboratories of Physiology in the Harvard Medical School

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There are several references in the recent literature to the influence of sympathetic denervation upon glycogen content of muscle. Hofmann and Wertheimer (1928) investigated the problem in frogs, and found no appreciable change in 1 to 2 days after (unilateral) sympathectomy. Büttner (1929) reported an increase in muscle glycogen after sympathectomy. From a series of observations on cats, Britton (1930) concluded that in the absence of sympathetic innervation muscle suffers a diminution in the glycogen content, and ascribed to the sympathetic nervous system a "glycogenotaxic" function. Beattie, Beattie and Milroy (1930) found no significant alteration in glycogen content of the affected muscles after lumbar sympathectomy in dogs.

In the present communication are presented data on the glycogen content of limb muscles in guinea pigs, cats and rabbits, at periods varying from 7 to 94 days after sympathetic denervation. The operations were performed under ether anesthesia; the animals were allowed to recover and were kept, for different periods, always in good condition. They were killed by cerebral concussion, and samples of muscle were rapidly excised and the glycogen analyses carried out by Pflüger's method.[3]

Preliminary glycogen determinations were made in the various limb muscles of normal animals, and also in animals subjected some time previously to anesthesia and sham operations. The results, given in table 1, show that there is not a wide discrepancy between the glycogen content of symmetrical limb muscles in any one individual—nor, indeed, between that of the muscles of upper and lower limbs.

In table 2 are shown the results of unilateral lumbar or upper thoracic sympathectomy, the glycogen percentage of the denervated muscles being

[1] Medical Fellow of the National Research Council.

[2] Fellow of the C. R. B. Educational Foundation.

[3] In cats and rabbits we took our samples always from the same group of muscles; but in the guinea pig, on account of the small size of the animal, we were obliged to remove as much of the musculature of the limb as possible without regard to particular muscles.

compared with that in the contralateral muscles. It will be seen that while sympathectomized muscle has, in most instances, a lower glycogen

TABLE 1

Percentage glycogen content of normally innervated limb muscles

ANIMAL	2 HIND LIMBS	2 FORE LIMBS	LEFT HIND LIMB	RIGHT HIND LIMB	LEFT FORE LIMB	RIGHT FORE LIMB
Guinea pig 1	0.24	0.21				
Guinea pig 2	0.34	0.26				
Guinea pig 3*	0.61	0.44				
Guinea pig 4			0.58	0.70		
Guinea pig 5			0.82	0.82		
Guinea pig 6			0.81	0.84		
Guinea pig 7					0.53	0.56
Guinea pig 8					1.53	1.25
Cat 9			0.36	0.33		
Cat 10			0.15	0.30		
Cat 11			0.19	0.19		

* 2 Hypogastric nerves severed.

TABLE 2

Effect of unilateral sympathectomy on muscle glycogen

ANIMAL	OPERATION—SYMPATHETIC CHAIN REMOVED	GLYCOGEN		DAYS AFTER OPERATION
		Denervated side	Normal side	
		per cent	*per cent*	
Guinea pig 24	Right abdominal	0.92	0.80	94
Guinea pig 25	Left abdominal	0.52	0.56	8
Guinea pig 26	Left abdominal	1.13	1.56	8
Guinea pig 27	Left abdominal	0.49	0.71	10
Guinea pig 28	Left abdominal	0.31	0.29	10
Guinea pig 29	Left abdominal	0.63	0.48	10
Guinea pig 30	Left abdominal	0.43	1.15	10
Guinea pig 31	Right upper thoracic	0.20	0.27	15
Rabbit 32	Right abdominal	0.77	0.72	7
Rabbit 33	Right abdominal	0.23	0.40	7
Cat 34	Right upper thoracic	0.22	0.37	10
Cat 35	Left upper thoracic	0.16	0.26	10
Average		0.50	0.63	

content, the difference is not often striking; nor, for that matter, is it regularly observed.

In another series of experiments sympathetic innervation to both upper or both lower limbs was destroyed and the glycogen content in the affected

muscles was compared with that of the limb muscles of the other extremity. As is shown in table 3, the sympathectomized muscles usually gave a decreased glycogen percentage, but the difference was not marked.

From our results it may be inferred that while exclusion of sympathetic nerve impulses to a region may be associated with a decrease in muscle glycogen, as Britton (1930) found, this decrease is neither very marked nor constant. The fact that sympathectomized muscles contain an appre-

TABLE 3

Muscle glycogen after bilateral sympathectomy

ANIMAL	OPERATION—SYMPATHETIC CHAINS REMOVED	GLYCOGEN		DAYS AFTER OPERATION
		Hind limbs	Fore limbs	
		per cent	*per cent*	
Guinea pig 12	Both abdominal	0.59	0.47	90
Guinea pig 13	Both abdominal	1.02	0.65	94
Guinea pig 14	Both abdominal	0.86	0.80	10
Guinea pig 15	Both abdominal	0.84	1.29	10
Guinea pig 16	Both abdominal	0.67	0.72	10
Guinea pig 17	Both abdominal	0.64	0.72	10
Rabbit 18	Both abdominal	0.10	0.14	7
Rabbit 19	Both abdominal	0.32	0.22	7
Cat 20	Both upper thoracic	0.35	0.30	10
Cat 21	Both upper thoracic	0.17	0.15	10
Cat 22	Both upper thoracic	Left, 0.36 Right, 0.33	Left, 0.27 Right, 0.34	10
Cat 23	Both upper thoracic	Left, 0.19 Right, 0.19	Left, 0.16 Right, 0.16	10

ciable amount of glycogen even months after denervation indicates that glycogen storage in muscle is not dependent upon sympathetic nerve impulses. Some of the observations of Hofmann and Wertheimer (1928) and of Beattie, Beattie and Milroy (1930) indicate that the sympathetic innervation may play a rôle in the rapid disappearance (utilization?) of stored glycogen during activity. That aspect of the problem requires further study.

BIBLIOGRAPHY

BEATTIE, F. J. R., M. B. BEATTIE AND T. H. MILROY. 1930. Journ. Physiol., lxix, 364.

BRITTON, S. W. 1930. This Journal, xciii, 213.

BÜTTNER, H. E. 1929. This Journal, xc, 304.

HOFMANN, A. AND E. WERTHEIMER. 1928. Pflüger's Arch., ccxviii, 176.

INSULIN AND HEART RATE AFTER SYMPATHECTOMY AND VAGOTOMY

SIMON DWORKIN[1]

From the Laboratories of Physiology, in the Harvard Medical School

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Using the denervated heart as indicator, Cannon, McIver and Bliss (1924) showed that hypoglycemia-inducing doses of insulin stimulated the sympathico-adrenal system to increased activity. Other observers have reported that insulin also stimulates parasympathetic nerves and nerve endings. Thus Bulatao and Carlson (1924) found after insulin an increase in rate and amplitude of gastric hunger contractions; this does not occur after section of the vagus nerves (Quigley and Templeton, 1930). In the pupil, a stimulating action of insulin upon parasympathetic nerves has been claimed by Poos and Risse (1926) and by Jorns (1927). In the salivary glands Ponirovsky and Ischunina (1929) stated that insulin not only stimulates salivary flow, but also accentuates the action of pilocarpine and is antagonistic to atropine.

The effect of insulin upon the (innervated) heart has been the subject of considerable research. The tachycardia usually noted after insulin—cited as one of the signs of insulin shock—is, according to the evidence in hand, to be attributed to sympathico-adrenal activity. On the other hand, slowing of the heart rate after insulin has been reported by several investigators (Garrelon and Santenoise, 1924; Haynal, 1925; Schaffer, Bucka and Friedlander, 1927; Lee, 1929). This slowing is generally ascribed to a vagotonic action. Further evidence of an apparent action of insulin on vagus fibres has been furnished by electrocardiographic studies (Wettgenstein and Mendel, 1924; Haynal, 1925; Okashima and Ohkuni, 1925; Haynal, Vidovsky and Gyorgyi, 1928). It is thus not unlikely that, as far as its influence upon the heart is concerned, insulin affects both divisions of the autonomic nervous system. The details of its action are not ascertained. Another problem that presents itself is that of correlating the increased gastro-intestinal activity after insulin (Bulatao and Carlson, 1924) with the excessive sympathetic activity and increased adrenin output (Cannon, McIver and Bliss, 1924).

The object of the present research has been to obtain more definite evidence concerning the action of insulin upon the heart, and, inferentially,

[1] Medical Fellow of the National Research Council.

upon the two divisions of the autonomic nervous system. Observations were carried out in three groups of animals. The first group comprised normal cats. The second group consisted of cats in which various portions of the heart nerves and the sympathico-adrenal system had been removed. The third group comprised completely sympathectomized cats, with heart denervated.

The technique evolved in this laboratory for the removal of the sympathetic nerve chains, and for the denervation of the heart has been described elsewhere (Cannon, Lewis and Britton, 1926; Cannon, Newton, Bright, Menkin and Moore, 1929). Most of the completely sympathec-

TABLE 1

Insulin and heart rate in normal cats

ANIMAL NUMBER	RESTING HEART RATE	INSULIN DOSE, UNITS PER KGM.	SLOWEST HEART RATE DURING HYPOGLYCEMIA	AMOUNT OF GLUCOSE INJECTED	HEART RATE AFTER 10 MINUTES
	per minute		*per minute*		*per minute*
Unanesthetized					
299	112–118	3.3	100–96	25 cc. 20% solution	130
300	124–130	4	100–106	25 cc. 20% solution	126–130
495		5	100–108	30 cc. 20% solution	160–180
496	86–92	5	100–108	40 cc. 25% solution	120–130
497	100–110	5	72–76	25 cc. 25% solution	92–100
297	76–82	6	84–92	30 cc. 25% solution	110–120
298	98–100	6	88–90	40 cc. 25% solution	130
499	180–190	4	180–192		
501	116–120	3.5	108–112	30 cc. 20% solution	144–150
Amytal anesthesia					
489	146–154	8	160–164	25 cc. 20% solution	167–176
491	184–190	1.7	205–208		
492	172–180	7.5	200–212	25 cc. 20% solution	212–230

tomized animals used in this research have been described in the last cited paper. The other operated animals were prepared in collaboration with Dr. R. M. Moore. At the time of use, the cats had completely recovered from the acute effects of the operations and to all intents were as vigorous and healthy as normal cats.

The basal heart rates were determined after the animals had been deprived of food for 16 to 18 hours, and had been resting quietly for 2 to 3 hours. Insulin was injected subcutaneously in doses to be mentioned later, and the heart rate observed (by stethoscope or by graphic means) for some hours. The observations were continued during the fall of the blood sugar, the ensuing convulsions and the subsequent injection of glu-

cose. The injection was made subcutaneously in every case. The blood-sugar changes were followed in some of the experiments by the micro-method of Folin (1928).

1. *Insulin hypoglycemia and heart rate in normal cats.* Inasmuch as the heart rate in the unanesthetized cat is subject to continual variations as the result of emotional excitement or movement, great care was taken to disturb the animals as little as possible during the experiments. The cats were kept in their accustomed cages throughout and the heart rate was counted by means of a stethoscope attached to the chest wall some

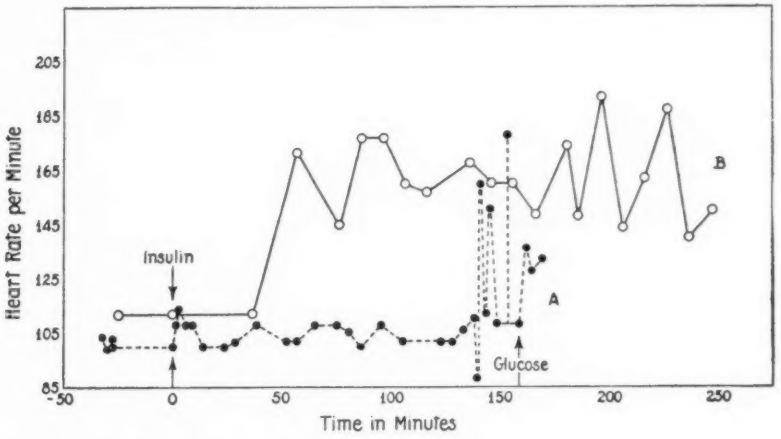

Fig. 1. A. The effect of insulin upon the heart rate in the normal cat (with vagus innervation present). Vertical lines represent rate during convulsions.

B. The effect of insulin upon the rate of the denervated heart (Cannon, McIver and Bliss, 1924).

hours previously. Excessive noises and other forms of stimulation were excluded as much as possible.

Nine cats were given insulin in amounts varying from 3.3 to 6 units per kilogram. The effect upon the heart rate is shown in table 1. Marked acceleration occurred in 2 cases; in the other experiments it was surprising to find that insulin, instead of accelerating the heart rate either did not alter it, or caused a noticeable slowing. In these latter instances a slight and very brief acceleration was often observed immediately after the injection; it lasted about ten minutes and can be ascribed to the attendant excitement. Thereafter the heart rate remained as a rule steady, until convulsions supervened. During convulsions, or as the result of outside stimulation, the heart rate became more rapid, but even this type of

acceleration usually subsided as the animal became quiet again. Sometimes during profound (quiet) hypoglycemia slower heart rates than any previously noted in the animals were obtained (fig. 1, A). Injection of glucose (amounts stated in table 1) promptly increased the rate by about 30 beats per minute, this acceleration being more or less permanent. Saline injections in equivalent amounts sometimes caused a small, temporary acceleration. In non-hypoglycemic animals, control experiments

TABLE 2

Insulin and heart rate after removal of portions of autonomic nervous system

ANIMAL NUMBER	BASAL HEART RATE	INSULIN DOSE, UNITS PER KGM.	HEART RATE DURING QUIET HYPOGLYCEMIA	AMOUNT OF GLUCOSE INJECTED	HEART RATE AFTER TEN MINUTES
	per minute		*per minute*		*per minute*
A. Adrenals denervated, heart nerves intact:					
No. 498	102–112	0.5	80–96	Atropin	140
No. 500	90–110	0.5	78–86	40 cc. 25% solution	120–136
	94–106	0.5	82–88	12 cc. 25% solution	116–120
	92–102	1.5	80–90	8 cc. 25% solution	116–120
No. 495	136–140	3	155–170	40 cc. 25% solution	176–180
B. Sympathetic chains removed: vagi intact:					
No. 417	116–122	1.3	94–100	30 cc. 25% solution	120–130
C. Vagus to heart removed, sympathetics intact:					
No. 416	140–152	2.5	158–166	60 cc. 25% solution	156–164
		3	148–168	30 cc. 25% solution	156–168
No. 299	146	4	150–162	50 cc. 25% solution	146–154
		4	190–200	40 cc. 25% solution	176–185
No. 268	118	4	164–180	30 cc. 25% solution	162–170

showed that glucose had little immediate effect on the rate of the heart (cf. Moore, 1929).

Edwards and Page (1924) as well as numerous other investigators have reported that under anesthesia insulin causes a marked increase in heart rate. Similar results were obtained in three of our experiments in which insulin was given after anesthetization with amytal (cats 489, 491, 492, table 1). It is likely that the uniformly fast heart rates noted in these experiments were due to suppression of vagus inhibitory effects by the anesthesia (cf. Shafer, Underwood and Gaynor, 1930).

2. *Insulin and heart rate after removal of parts of the autonomic nerves.*

a. In one animal (cat 417, table 2) the sympathetic nerve chains were excised. As far as nervous control was concerned, the heart was subject only to vagus impulses. This animal received 1.3 units insulin per kgm. Here also the relatively slow heart rate, found in the normal cat after insulin, was observed. Glucose caused a prompt acceleration of about 30 beats per minute.

b. The effect of insulin hypoglycemia upon the rate of the heart from which direct nervous impulses have been excluded, but which is still subject to sympathico-adrenal activity has been studied by Cannon, McIver and Bliss (1924). As the blood sugar falls to a critical level, a marked cardiac acceleration occurs, which can be checked by administering glucose. This observation was repeated in cat 268 (table 2) with similar results.

In several animals the sympathetic nerves to the heart as well as to the adrenal glands were left intact. Vagus innervation to the heart was excluded by sectioning of the right vagus below the recurrent laryngeal branch, and cutting of the cardiac branches of the left nerve. After recovery, it was found that the heart rate of most of these animals was so fast and variable—even after prolonged rest—as to make observations with insulin unreliable. However, in 2 cats (416, 299, table 2) in which "basal" rates as slow as 140 to 150 per minute were noted, insulin was followed by considerable acceleration. No marked or prompt reduction in rate occurred after injection of glucose.

c. A few observations were made in cats in which central sympathetic impulses to the adrenal glands as well as to the liver were prevented by extirpation of the abdominal sympathetic chains (cats 498, 495, 500, table 2). The innervation of the heart was left intact. It has been shown by Cannon, McIver and Bliss (1924) and by Britton, Geiling and Calvery (1928), that such animals are hypersensitive to insulin. These experiments were therefore carried out with small doses of insulin (0.5 to 1 unit per kgm.). As table 2 shows, in 4 instances out of 5 the heart rate during profound hypoglycemia was slower than the basal rate. Indeed, the figures obtained were considerably slower than those noted in animals with adrenal innervation intact, under similar conditions. The additional slowing may very well be attributed to the absence of medulli-adrenal secretion. Glucose had an effect similar to that seen in normal animals.

Figure 2 shows records of the heart rate in the conditions mentioned, during quiet hypoglycemia, and after glucose administration.

The effect of hypoglycemic doses of insulin upon the rate of the heart innervated by the vagus nerve is, evidently, different from that observed in the heart subject to sympathico-adrenal stimulation alone. The heart rates under the two conditions are represented in figure 1. The curve marked *B* is taken from the paper of Cannon, McIver and Bliss (1924).

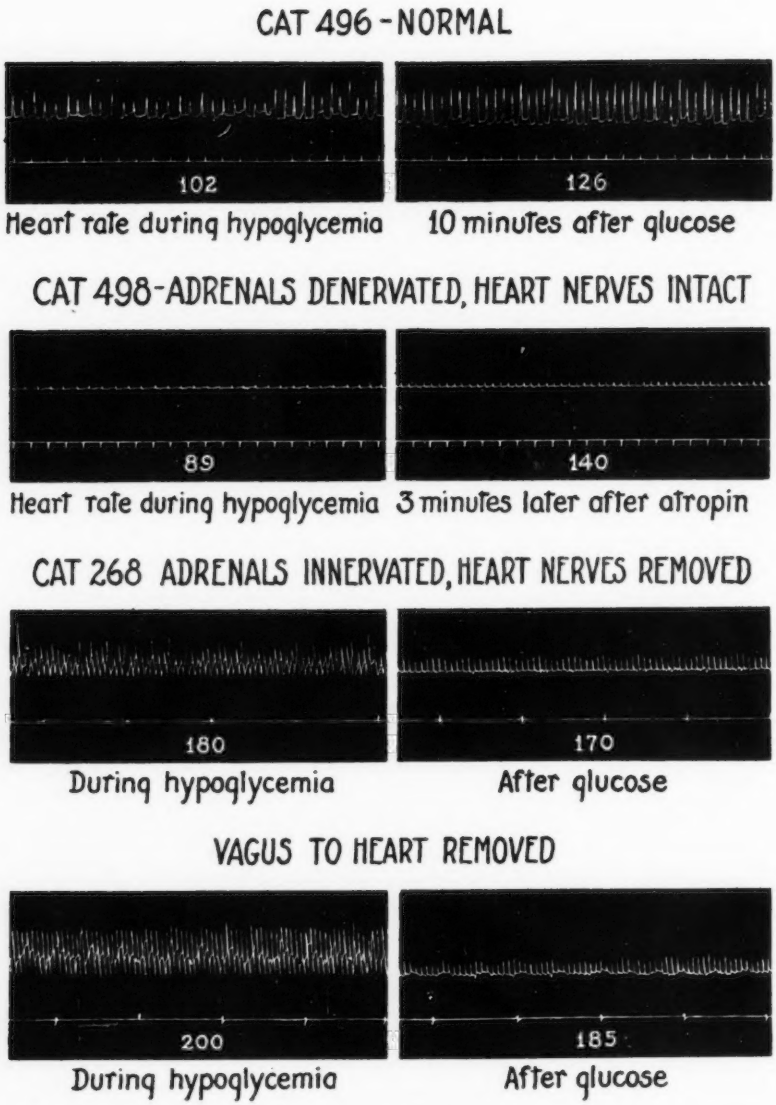

Fig. 2. Heart rates of cats during hypoglycemia, and after subsequent glucose injection.

It would seem that, ordinarily, the vagus nerves as well as the sympathetic system are stimulated to increased activity after insulin, and that the vagus influence predominates, for it has been shown that the rate of the heart with vagus innervation is never as fast as that of the heart from which vagus impulses have been excluded. In the former condition the rate seldom exceeds 110 beats per minute (except during convulsions) while in the latter condition the rate is usually above 160 and may exceed 200 per minute. It would seem, too, that the observed insulin vagotonia is of central origin.[2]

3. *Insulin and heart rate in completely sympathectomized cats with heart denervated.* Several observations were made on cats in which, after removal of both sympathetic chains from the stellate ganglion to the pelvis, the vagus banches to the heart were cut. The heart in such an animal is isolated as far as central nervous influences are concerned, but is still subject to the influence of chemical agents brought to it in the blood stream (Cannon, Lewis and Britton, 1926). Moore (1929) has found a prolonged, marked acceleration following a protein meal.

These cats were found to be extremely sensitive to insulin; doses as small as 0.2 to 0.5 unit per kgm. lowered the blood sugar to the convulsive level in less than two hours. A more detailed account of the action of insulin in completely sympathectomized animals will be given in another communication

In 5 experiments out of 14 a definite slowing of the heart rate occurred during hypoglycemia (cats 384, 396, table 3). In several instances in which the cats were allowed to go into convulsions a much more marked slowing was observed, associated perhaps with asphyxia due to spasm of the diaphragm. The rest of the animals were given glucose after the manifestation of pre-convulsive signs. Their condition soon returned to normal. The heart rate, however, usually became progressively slower, so that 3 to 4 hours after the insulin injection (and 30 to 90 minutes after receiving glucose) the rate of the heart was considerably below the basal: Furthermore, in some cases skipped beats and extra-systoles were noted at this time. Restoration of the normal rate and rhythm could always be effected by injection of atropin (1 mgm. per kgm. atropin sulphate subcutaneously). Glucose injected without previous insulin administration had little effect on the heart rate. The slowing must, therefore, be regarded as a late effect of insulin hypoglycemia.

It is apparent that insulin is capable of reducing the rate of the heart when the latter is no longer subject to central vagus influences—provided that the effect of the sympathetic system is excluded. The slowing may be

[2] Recently La Barre and Destrée (1930) have intimated that the gastric hypermotility induced by insulin hypoglycemia is controlled by the activity of parasympathetic centres in the region of the thalamic nuclei.

due to a direct action of insulin (or of hypoglycemia) upon the heart muscle, or upon the post ganglionic vagal neurons. The results of atropine injection are suggestive of the latter influence.

The modifications of heart rate after insulin observed in these animals are obviously different from those described previously. In the first place, it is apparent that in comparison with the central action, the peripheral action of insulin is a weak one, and does not counteract the effects of sympathico-adrenal stimulation (cf. cat 268, table 2). Secondly, in the presence of the vagus nerves, glucose given to a hypoglycemic animal is

TABLE 3

Insulin and heart rate in completely sympathectomized cats, with heart denervated

ANIMAL NUMBER	BASAL HEART RATE	INSULIN DOSE, UNITS PER KGM.	HEART RATE DURING QUIET HYPOGLYCEMIA	AMOUNT OF GLUCOSE INJECTED	HEART RATE AFTER 1 HOUR
	per minute		*per minute*		*per minute*
384	102–105	0.3	88–94	15 cc. 20% solution	83–85
	104–109	0.3	87–94	16 cc. 25% solution	88
	103–110	0.3	86–92	Atropin	109
391	90–95	0.2	103–107	20 cc. 25% solution	86–88
	105–112	0.2	108–115	16 cc. 25% solution	102–105
396	100–102	0.15	96	15 cc. 20% solution	86–76
	108	0.15	66–90	15 cc. 20% solution	Arrhythmia
	93–120	0.25	118–120	15 cc. 20% solution	Arrhythmia
400	103–105	0.4	103–108	15 cc. 20% solution	88–91
	103–110	0.2	102	20 cc. 20% solution	88–91
403	90–92	0.2	87–90	20 cc. 20% solution	76–78
	87–88	0.2	81–83	15 cc. 20% solution	79–80
	92–98	0.2	96–102	15 cc. 20% solution	86
404	102–109	0.3	107–108	20 cc. 20% solution	96–100

always followed by cardiac acceleration. Thirdly, the sudden sharp increase in rate of the heart subject to central nervous impulses (or increased adrenin secretion) during hypoglycemic convulsions does not occur in completely sympathectomized cats.

A comparison of the effects of insulin on the heart rate in these cats with those observed in the isolated heart, or the heart-lung preparation is of interest. The latter question has been studied by many investigators. In the frog heart, Collazo and Handel (1923) found that insulin had no effect upon the size or frequency of contractions. Citron and Zondek (1924) as well as Kogan (1924) observed a prompt slowing, and concluded that in the heart the insulin effect is analogous to the vagus effect. Frey (1925) and Beskow (1926) pointed out that large concentrations of in-

sulin in the perfusing fluid caused a decrease in heart activity, but that moderate, or small amounts had no effect. In the isolated mammalian heart, the early experiments of Hepburn and Latchford (1922) showed no definite effect of insulin. Burn and Dale (1924) found that addition of insulin caused the heart to beat more rapidly and vigorously. Kogan (1924) and Rigoni and Stella (1926) noted that, as in the case of the frog heart, large concentrations of insulin had a depressant action, while smaller amounts had little effect. Peserico (1926) found, in several instances, a slowing of the heart after insulin. But investigations with the heart-lung preparation show clearly that moderate or small amounts of insulin have no definite influence upon the rate or amplitude of the heart beat (Plattner, 1925; Visscher and Müller, 1926; Bayliss, Müller and Starling, 1918).

It must be pointed out, however, that the experiments just cited have been concerned primarily with the effect of insulin and glucose, rather than hypoglycemia. As a rule, during the course of these researches, glucose was added to the circulating fluid as it was being used up, so that there was always a surplus of sugar rather than anything approaching hypoglycemia. No systematic study of the effect of hypoglycemia upon the isolated heart seems to have been made. Plattner (1924) states that in two of his experiments insulin was allowed to act upon the heart without glucose; the results were not different from the others obtained by him.

SUMMARY AND CONCLUSIONS

The action upon the heart rate of hypoglycemia-inducing doses of insulin was tested in normal cats, and in cats surviving excision of portions of the autonomic nervous system. The following changes may occur: 1, acceleration, due to central sympathico-adrenal impulses; 2, slowing, due to central vagus stimulation; 3, slowing, due to a peripheral action. In the intact animal these effects are expressed in a heart rate that is seldom above the basal level. The inference is that, while both divisions of the autonomic nervous system may be stimulated during insulin hypoglycemia, it is the vagus influence that predominates in organs that are innervated by both sets of nerves. These observations are in accordance with the findings of Bulatao and Carlson (1924) and correlate them with the work of Cannon, McIver and Bliss (1924). Conclusions very similar to these, regarding the action of insulin upon the gastro-intestinal tract, have been arrived at by Quigley and Templeton (1930).

In completely sympathectomized cats with heart denervated insulin has a delayed action upon the heart rate, manifested in a prolonged, though not marked slowing. This slowing develops gradually, attaining the maximum long after the lowest blood-sugar level has been reached, and some time after injection of glucose. Injected without previous insulin administration, glucose has no definite effect on heart rate in these animals. The slowing can be promptly abolished by atropine sulphate.

My thanks are due to Prof. W. B. Cannon, at whose suggestion the problem was undertaken, for his interest and helpful criticism.

BIBLIOGRAPHY

BAYLISS, L. E., E. A. MÜLLER AND E. H. STARLING. 1928. Journ. Physiol., lxv, 33.

BESKOW, A. 1926. Skand. Arch. f. Physiol., xlvii, 127.

BRITTON, S. W., E. M. K. GEILING AND H. O. CALVERY. 1928. This Journal, lxxxiv, 141.

BULATAO, E. AND A. J. CARLSON. 1924. This Journal, lxix, 107.

BURN, J. H. AND H. H. DALE. 1924. Journ. Physiol., lix, 164.

CANNON, W. B., J. T. LEWIS AND S. W. BRITTON. 1926. This Journal, lxxvii, 326.

CANNON, W. B., M. A. MCIVER AND S. W. BLISS. 1924. This Journal, lxix, 46.

CANNON, W. B., H. F. NEWTON, E. M. BRIGHT, V. MENKIN AND R. M. MOORE. 1929. This Journal, lxxxix, 84.

CITRON, J. AND S. G. ZONDEK. 1924. Klin. Wochenschr., iii, 1243.

COLLAZO, J. A. AND M. HANDEL. 1923. Deutsch. Klin. Wochenschr., xlix, 1546.

EDWARDS, D. J. AND I. H. PAGE. 1924. This Journal, lxix, 177.

FOLIN, O. 1928. Journ. Biol. Chem., lxxvii, 421.

FREY, E. 1925. Arch. f. exp. Path. u. Pharm., cv, 343.

GARRELON, L. AND D. SANTENOISE. 1924. Compt. Rend. Soc. Biol., xc, 470.

HAYNAL, E. 1925. Klin. Wochenschr., iv, 403: 1729.

HAYNAL, E., L. VIDOVSKY AND C. GYORGYI. 1928. Klin. Wochenschr., vii, 1543.

HEPBURN, J. AND J. K. LATCHFORD. 1922. This Journal, lxii, 177.

JORNS, G. 1927. Zeitschr. f. ges. exp. Med. liv, 179.

KOGAN, V. M. 1924. Zeitschr. f. ges. exp. Med., xlii, 25.

LA BARRE, J. AND P. DESTRÉE. 1930. Compt. Rend. Soc. Biol., civ, 112.

LEE, M. O. 1929. Proc. XIII Internat. Congress, Boston. This Journal, 1929, xc, 427.

MOORE, A. R. 1929. This Journal, lxxxix, 515.

OKASHIMA, K. AND J. OHKUNI. 1925. Journ. Orient. Med., iii, 32.

PESERICO, E. 1926. Arch. di Fisiol., xxiii, 488.

PLATTNER, F. 1924. Journ. Physiol., lix, 289.

PONIROVSKY, N. AND W. ISCHUNINA. 1929. Proc. XIII Internat. Congress, Boston. This Journal, 1929, xc, 476.

POOS, F. AND O. RISSE. 1926. Arch. f. exp. Path. u. Pharm., cxii, 176.

QUIGLEY, J. P. AND R. D. TEMPLETON. 1930. This Journal, xci, 482.

RIGONI, M. AND G. STELLA. 1926. Arch. di Fisiol., xxiv, 293.

SCHAFFER, H., E. BUCKA AND K. FRIEDLANDER. 1927. Zeitschr. f. ges. exp. Med., lvii, 35.

SHAFER, G. D., F. J. UNDERWOOD AND E. GAYNOR. 1930. This Journal, xci, 461.

VISSCHER, M. B. AND E. A. MÜLLER. 1926. Journ. Physiol., lxii, 341.

WETTGENSTEIN, A. AND B. MENDEL. 1924. Klin. Wochenschr., iii, 1119.

IMPOTENCE OF THE MALE RODENT AFTER SYMPATHETIC DENERVATION OF THE GENITAL ORGANS

Z. M. BACQ[1]

From the Laboratories of Physiology in the Harvard Medical School

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In connection with the study of sympathectomized animals carried out by Cannon and his collaborators, it was thought that it would be interesting to take up again the question of the sympathetic innervation of the male genital organs. It is a generally accepted idea that ejaculation is controlled by a sympathetic center situated in the lower part of the medulla, but the evidence one can cull from the literature is contradictory concerning the relation between erection and ejaculation. The method of electrical stimulation has given nearly all the available information (Remy, 1886; Sherrington, 1892; Langley, 1895; Akutsu, 1903). The results of this method I shall discuss later in comparison with my results. I have used only acute or long lasting sympathectomized preparations.

The present study is confined to rodents (guinea pigs, rats and rabbits) for several reasons: the time of gestation in these animals is short; their sexual activity is great and not affected by their restraint in the laboratory; and anatomically and physiologically their sexual organs are less complicated than those of cats and dogs. But the study of sympathectomized cats and dogs has not brought any evidence which may be considered as contradictory to the observations reported in the present paper.

I. Acute experiments. Spina (1897) has shown that if the spinal cord of a male guinea pig is transected between D_{12} and L_1, spontaneous erection occurs, followed by expulsion of semen. I tried by severance of various sympathetic fibres to change this reaction. The preliminary directions detailed by Spina (1897) were found perfectly exact, and I had only to follow them closely.

The procedure was as follows. Different series of vigorous, full-grown males were chosen. In acute experiments the animals were isolated from the females during four to seven days before the experimental test. In the experiments made in two stages no sexual contact was allowed after the preliminary operation. In order to release the genital reflex, the skin was incised at the union of the thoracic and lumbar regions under light ether anesthesia; a knife was introduced into the vertebral canal at the level of

[1] Fellow of the C. R. B. Educational Foundation.

the last costo-vertebral articulation and the spinal cord was transected. Ether anesthesia was then stopped. Quickly the animal was fastened on his back to the board and the prepuce was divided so as to allow better observation. In a normal animal, from one to seven minutes after transection, rhythmic movements occur in the anogenital region; the penis becomes longer and thicker; the glans has the aspect of a fan divided in two lobes; the two horny appendices are protruded. It is a complete erection which ends by the discharge of a vitreous mass, the semen. Sometimes it was found necessary to stimulate slightly the mucous membrane of the glans in order to release the phenomenon.

The sympathetic innervation of the internal genital organs of the guinea pig was described first by Remy (1886), later and more accurately by

TABLE 1

Spina's experiment. Transection of spinal cord immediately after sympathectomy

ANIMAL NUMBER	OPERATION	TIME ELAPSED BETWEEN TRANSECTION OF SPINAL CORD AND BEGINNING OF REFLEX	ERECTION	EJACULATION
		minutes		
10	Control	7	Complete	+
13	Control	1	Complete	+
9	2 abdominal chains removed from pelvic ganglion to second lumbar ganglion inclusive	2	Slight	0
14	Abdominal sympathectomy	1	Slight	0
12	Hypogastric nerves severed	2	Slight	0
11	Abdominal sympathectomy and severance of two hypogastric nerves	2	Slight	0

Akutsu (1903). A good picture of the innervation of the seminal vesicles and vasa deferentia is to be found in Akutsu's paper. Sympathetic fibres run from the second, third, fourth and fifth lumbar ganglia to the inferior mesenteric ganglion (also called by certain authors, hypogastric ganglion). From the inferior mesenteric ganglion, two nerves, the hypogastrics, run in the mesentery to the hypogastric plexus, at the base of the seminal vesicles. According to Akutsu (1903) the cell bodies of the outlying neurones are situated in the inferior mesenteric ganglion, but a second connection should exist in the hypogastric plexus. In a first series of experiments, transection of the spinal cord was performed immediately after abdominal sympathectomy, or severance of the hypogastric nerves. Results given in table 1 show that ejaculation never occurred. But as a

general rule, the erection in this series of experiments was definitely slighter, and I therefore adopted another more satisfactory technique.

In a first sterile operation, abdominal sympathectomy was carried out under ether; and the spinal cord was transected only when the animals had recovered and were in perfect condition, i.e., five to seven days after the operation. Sham operation was performed on a few control animals. The animals which were found in poor condition were discarded.

Results of this series of experiments are given in table 2. Without exception, erection, sometimes slighter than normal, occurred at the required time, but it was never followed by ejaculation. As far as the

TABLE 2

Spina's experiment, 5 to 7 days after complete sympathetic denervation of the genital organs

ANIMAL NUMBER	OPERATION	TIME ELAPSED BETWEEN TRANSECTION OF SPINAL CORD AND BEGINNING OF REFLEX	ERECTION	EJACULATION	REMARKS
		minutes			
21	Control	1	Complete	+	
15	Abdominal sympathectomy	6	Big	0	
19	Abdominal sympathectomy	1	Big	0	
22	Abdominal sympathectomy	3	Complete	0	
16	2 hypogastric nerves severed	1	Big	0	
17	2 hypogastric nerves severed	2	Complete	0	
18	2 hypogastric nerves severed	1	Big	0	Drop of urine expelled at the end of erection

movements of the perineal region were concerned, I have been unable to observe the slightest difference between sympathectomized and control animals. Furthermore, as should be expected, severance of the hypogastric nerves (post-ganglionic fibres) or removal of the two abdominal chains (severance of preganglionic fibres) gave quite similar results.

The question arises whether the sympathetic system must be intact in order to obtain the ejaculation of semen. It is well known that the sympathetic system generally works as a whole, and since sexual emotion evokes a considerable sympathetic excitement, it was of interest to repeat Spina's experiment in animals, the genital organs of which had been only

partially denervated. For this purpose, in a preliminary operation in two animals, I severed one hypogastric nerve, and in three others I cut only the rami going from one or two ganglia to the inferior mesenteric ganglion, as indicated in table 3. Ejaculation after transection of the spinal cord at the usual level did not happen, or it was not complete: in two cases the semen remained in the penis; in the other cases, just a drop of prostatic secretion or urine was forced out.

TABLE 3

Spina's experiment after partial sympathetic denervation of the genital organs

ANIMAL NUMBER	OPERATION	TIME ELAPSED BETWEEN TRANSECTION OF SPINAL CORD AND BEGINNING OF REFLEX	ERECTION	EJACULATION	REMARKS
		minutes			
20	Left hypogastric nerve severed	1	Complete	0	One drop of prostatic secretion expelled at the end of erection
26	Right hypogastric nerve severed	2	Complete	±	Semen in the penis, not spontaneously expelled
27	Section of rami from second left lumbar ganglion to inferior mesenteric ganglion	2	Complete	±	Semen in the penis
28	Section of rami from second and third left lumbar ganglion to inferior mesenteric ganglion	2	Complete	0	Drop of prostatic secretion expelled at the end of erection
29	Section of rami from third left lumbar ganglion to inferior mesenteric ganglion	1	Complete	0	Drop of urine expelled

Thus, if we consider Spina's experiment as a kind of artificially produced coitus, we may conclude that removal of the sympathetic innervation to the genital organs of the male guinea pig prevents ejaculation, but not erection; also that partial denervation may seriously impair the mechanism of ejaculation. If that is true, sympathectomized males should be able to copulate, but not to reproduce. That inference has been investigated in rats, rabbits and guinea pigs.

II. Long lasting experiments. In order to make the experiments more demonstrative, the animals were operated on at the beginning of

spring, a season during which sexual activity of rodents is great. The date of fecunation was calculated backwards on the basis of 21 days of gestation for the rat, and 68 for the guinea pig. In certain cases, we were obliged to sacrifice the females during pregnancy; the age of the fetus was judged by the weight, and the date of fecundation calculated approximately.

a. *Rats.* The animals used in these experiments were from the stock of the Wistar colony. The males were in perfect condition after the operation—gaining in weight and indistinguishable from normal males. Care was taken to avoid avitaminosis by giving a diet of mixed grains, whole bread and milk, and fresh vegetables. As stated below, the ability of the females to reproduce was tested in several instances in allowing them sexual relations with normal males. Furthermore, we used as a control one male from which only a small part of the sympathetic innervation to the genital organs had been removed.

Our five observations on the rat are as follows:

Rat 6. Ten months old. Two abdominal sympathetic chains removed under ether from the pelvic ganglion to the second lumbar ganglion inclusive. With three young females during 30 days. No pregnancy. Two of the females at the end of the experiment were allowed sexual relation with normal males and gave birth to normal litters of 7 and 9 young.

Rat 34. Four months old. Bilateral abdominal sympathectomy. With three young females during 6 months. No pregnancy. Killed at the end of experiment; body weight, 336 grams; weight of testes, 3.3 grams.

Rat 23. Four months old. Bilateral abdominal sympathectomy. With three young females during 6 months. No pregnancy, except in one case: female 32, 51 days after the operation on the male, gave birth to the two young only. Fecundation occurred one month after the operation and may be attributed to the fact that at the end of copulation, sympathectomized males sometimes expel a drop of urine in which living spermatozoa may be present.[2] Killed at the end of the experiment. Body weight, 340 grams; weight of testes, 3.4 grams.

Rat 22. Four months old. Right abdominal sympathetic chain removed. With three young females during 6 months. No pregnancy. One of these females, when allowed sexual relations with a normal male at the end of the fifth month of the experiment, gave birth to a normal litter of 5 young.[3] Killed at the end of experiment. Body weight, 378 grams; weight of left testis, 1.62 grams, of the right one, 0.85 gram.

Control rat 38. Four months old. Two abdominal sympathetic chains removed from the promontory to the third lumbar ganglion exclusive. With three females.

[2] Similar observations on guinea pigs have been made by me in acute experiments and also by Remy (1886); the unusually small size of the litter is in favor of the explanation given in the text.

[3] It should be noted here that removal of one abdominal chain in the rat is impossible without injury to the other chain on account of the peculiar anatomical disposition of the lumbar sympathetic system in that animal: the chains are separate, but at the level of each ganglion they are connected across the middle line, and a kind of artificial dissection must be made for unilateral sympathectomy. In any case, however, the genital organs of rat 23 were only partially denervated.

Two of them littered (8 young in each case); fecundation occurred in one case 13 days, in the other 23 days, after the operation on the male.

Thus three male rats, the internal genital organs of which had been completely denervated, were impotent, two of them for a period of 6 months. The control rat recovered its potency very soon after the operation, but rat 23 was permanently impotent after removal of one abdominal chain only. This last observation will be discussed later. The sexual behavior of these animals was normal. Two days after sympathectomy they were already following the females and copulating. It is impossible to tell whether the erection was complete or not. Further experiments on rabbits were much more demonstrative on that point.

b. *Rabbits.* At the beginning of spring (March 24) I removed the two abdominal sympathetic chains (from the pelvic ganglion up to the splanchnics inclusive) from two full-grown male rabbits under somnifene anesthesia (0.5 cc. per kgm., intravenously). I noticed at the end of the operation a very slight flaccid erection.

Two days after the sympathectomy, the animals were in good condition but refused to copulate. The next day, however, when put in a large cage with two females, both of them became very excited and readily copulated. This copulation differed very much from the normal; it was prolonged, the characteristic final orgasm, so obvious in the normal male (the animal utters a cry and falls on his side), never happened; and never could I find semen in the vagina. After a while the females did not accept these impotent males, but were chased by them until stopped by fatigue. During their sexual excitement, the sympathectomized males exhibited a prolonged erection; the penis was stiff but the tip was shaped like a hook; a slight pressure at the base of the organ or on the abdominal wall readily caused it to become straight.

Two weeks after the operation, the males, isolated previously for four days, exhibited the same kind of incomplete copulation. One month after the operation, the introduction of a new female into the cage was the signal for great sexual excitement. The males continued chasing the female during more than half an hour until both males and female lay panting, exhausted. Outside of the periods of sexual excitement, no erection was observed. At the beginning of June, one of the two females was pregnant. When the animal was killed three fetuses were found in the uterus; fecundation occurred in that case about 45 days after the operation on the male. This fact will be discussed later.

In the fall, we tried to repeat these observations, but without success. The males were not in such good condition after the operation and refused to copulate.

c. *Guinea pigs.* Vigorous, healthy animals were used in these experiments. They were kept in large cages. The diet consisted of oats, hay,

fresh vegetables, oranges and water. Milk also was given from time to time. As a control animal, we used a male in which only the right abdominal chain had been removed.

Guinea pig 277. Bilateral abdominal sympathectomy from the pelvic ganglion up to the second lumbar ganglion inclusive. In very good condition during 4 months. Died from pneumonia 5 months after the operation. During 5 months with four females. No pregnancy.

Guinea pig 34. Bilateral abdominal sympathectomy. With three females during 3 months. One of them gave birth to four young; fecundation occurred 37 days after the operation of the male. Another had a litter of two young; fecundation occurred 38 days after the operation.

Post-mortem examination. Fibres traced from the second right lumbar nerve to the hypogastric ganglion; chains well removed.

Guinea pig 33. Bilateral abdominal sympathectomy. With three females during 3 months. When killed at the end of the experiment, two of them were found pregnant (three fetuses in each case); fecundation occurred about two months after the operation on the male.

Post-mortem examination. On the right side, sympathetic fibres traced from the second and third lumbar nerves to the inferior mesenteric ganglion; chains well removed.

Guinea pig 30. Inferior mesenteric ganglion removed with long piece of hypogastric nerve. With four females during three months. One of them only, when killed at the end of the experiment, had one fetus in the left uterine horn. This case is to be correlated with that of rat 23; probably accidental fecundation by urine, as indicated by the unusually small size of the litter.

Post-mortem examination. No detectable regeneration.

Control guinea pig 32. Right abdominal chain removed. With three females during three months. Two of them littered. Fecundation occurred in one case 13 days after the operation of the male, in the other, 22 days. The third female was found pregnant when killed at the end of the experiment. Post-mortem examination: weight of the right seminal vesicle with contents, 3.9 grams, the left one, 2.1 grams.

Post-mortem examination revealed in each case a tremendous enlargement of the denervated seminal vesicles. The lower abdomen was entirely occupied by these distended organs (confirming Remy's observations, 1886). In animal 32 the weight of the denervated seminal vesicle was nearly double that of the normal one. A similar phenomenon, but on a smaller scale, has been reported (Bacq, 1930) after abdominal sympathectomy of the rat. If the observation on guinea pig 277, which was not perfect, is discarded, it is clear that bilateral abdominal sympathectomy resulted in temporary impotence. However, an animal from which the postganglionic sympathetic neurones innervating the genital organs had been removed was definitely impotent. Unilateral sympathectomy did not affect visibly the ability of another male to partake in reproduction.

The sexual behavior of sympathectomized guinea pigs seems normal. They copulate and are very jealous and quarrelsome whenever another male is introduced in their cage. I was unable to observe the prolonged erection seen in sympathectomized rabbits during sexual excitement.

III. Discussion. Remy (1886) and Akutsu (1903) in the guinea pig and Langley and Anderson (1895) in the rabbit and the cat have obtained ejaculation without erection by electric stimulation of the sympathetic preganglionic or postganglionic fibres innervating the genital organs. That sympathectomy results in absence of ejaculation without disappearance of erection was to be expected. But our long lasting experiments show further facts which call for discussion.

Why was it that after bilateral abdominal sympathectomy, two guinea pigs were only temporarily impotent? During the operation, only the preganglionic fibres were cut. It is well known (c.f. Langley, 1897–1900) that preganglionic fibres regenerate easily; Bacq and Dworkin (1930) found also anatomical and physiological evidence of regeneration of these fibres in the cat; and post-mortem examination of our temporarily impotent guinea pigs revealed unquestionable regeneration of the fibres going to the inferior mesenteric ganglion. That extirpation of the hypogastric ganglion in another guinea pig resulted in permanent impotence, is definite evidence of the soundness of this explanation; for, in that case, functional regeneration was prevented by removal of the postganglionic cells[4] (Langley, 1900). Regeneration also happened in rabbits 45 days after abdominal sympathectomy. The permanent impotence of the sympathectomized rats is puzzling. We do not know where the postganglionic cells of the hypogastric nerves are located in the rat; we assume that they are not situated in the abdominal chains. Without question regeneration of preganglionic fibres occurs in this animal as well as in the other mammals; on account of their small size, we cannot verify this fact for the rami going to the inferior mesenteric ganglion, but some observations made on the splanchnics do not leave any doubt (Bacq, 1930). Three explanations seem possible: first, it may be that the rat needs a practically intact sympathetic innervation in order to be able to act in reproduction (the observation on rat 22, permanently impotent after unilateral abdominal sympathectomy, is in favor of this hypothesis, especially when compared with the observation on guinea pig 32 which after the same operation was perfectly potent); secondly, Langley and Anderson (1895) have shown that in the rabbit and the cat, unilateral stimulation of the lumbar roots causes contraction of both seminal vesicles, and this crossed innervation may be very important in the rat[5]; thirdly, prostatic secretion may be abolished in the rat and fail to produce its fertilizing action on the spermatozoa.

Takahashi (1922a) and Marconi (1923) have observed (the former in

[4] Remy (1886) also recorded a permanent impotence in guinea pigs after removal of a long piece of the two hypogastric nerves.

[5] This crossed innervation probably exists also in the guinea pig, because the enlargement of the seminal vesicle is more marked after bilateral than after unilateral sympathectomy.

guinea pigs, the latter in dogs) atrophy of the testicles after abdominal sympathectomy or removal of the inferior mesenteric ganglion. Lipschütz and Voss (1924) have not observed any change in the testicles of the cat after removal of the two lowest lumbar ganglia, but their experiments are not conclusive since the operation leaves intact the innervation of the genital organs. According to Takahashi (1922b) the degeneration involves chiefly the seminal epithelium. Such an atrophy did not occur in our guinea pigs, since they recovered potency after regeneration of the preganglionic fibres. The weight of the testicles of our impotent rats was perfectly normal, except in one case (rat 22), and careful histological examination showed no degeneration at all of the seminal epithelium, even in the right testicle of rat 22 which weighed only half of the normal weight; indeed, these testicles were full of apparently normal spermatozoa.

Remy (1886) states that his impotent guinea pigs could not have a complete erection during sexual excitement. On the other hand, it has been noticed by Takahashi (1922 a) and others that sympathetic denervation of the genital organs resulted in continuous erection, and this fact was explained by an antagonistic action between sympathetic and parasympathetic systems, the action of the latter being predominant after exclusion of the former. What we have observed both in long lasting and acute experiments is that erection happens only during sexual excitement or after transection of the spinal cord.[6]

Remy (1886) and Akutsu (1903) have observed that repeated electric stimulation of the hypogastric nerves of the guinea pig slowly induces erection and hypersensitivity of the mucous membrane of the glans. I have observed (table 2) that after transection of the spinal cord, erection in sympathectomized animals may not be as complete as it is in normal animals. This, however, is a very secondary phenomenon, since the long lasting preparations have shown unquestionably that erection is still induced by sexual excitement after sympathectomy.

The absence of ejaculation is thus the regular and important deficiency after exclusion of the sympathetic innervation of the genital organs of the male rodent; it is due to the disappearance of the contraction of the vasa deferentia and the seminal vesicles, and also probably to the lack of prostatic secretion.

SUMMARY AND CONCLUSION

1. If the spinal cord of a male guinea pig isolated from the females during a few days is transected between T_{12} and L_1, rhythmic movements occur in the ano-genital region, followed by erection and ejaculation (Spina, 1897). If the same experiment is repeated after abdominal sympa-

[6] A very few of our operated animals exhibited during one or two weeks a kind of incomplete, flaccid erection.

thectomy or severance of the two hypogastric nerves, rhythmic movements and erection occur, but ejaculation is always absent. Partial sympathetic denervation also abolishes or impairs this reflex ejaculation.

2. Bilateral abdominal sympathectomy induced permanent impotence in two young rats; the same result was obtained in another after unilateral sympathectomy.

Temporary impotence was observed after bilateral abdominal sympathectomy in two guinea pigs. Permanent impotence was recorded in another one after removal of the hypogastric nerves and inferior mesenteric ganglion (confirming Remy's observations, 1886). This difference is due to the regeneration of the preganglionic fibres.

3. Sexual behavior of the sympathectomized rodents is described.

4. Remarkable enlargement of the denervated seminal vesicles was observed in the guinea pig (confirming Remy's findings, 1886). No atrophy whatsoever could be detected in the testes of our impotent rats. No persistent erection was observed after sympathetic denervation of the internal genital organs.

5. Our general conclusion is that the presence of the sympathetic nerve supply to the male genital organs is necessary for ejaculation and consequently for reproduction.

We wish to express our thanks to Doctor Cannon for his keen interest in this work and for his frequent helpful advice. We are also indebted to Mr. Pinkston for careful histological preparations.

BIBLIOGRAPHY

AKUTSU, S. 1903. Pflüger's Arch., xcvi, 541.
BACQ, Z. M. 1930. This Journal, xcv, 601.
BACQ, Z. M. AND S. DWORKIN. 1930. This Journal, xciii, 629.
LANGLEY, J. N. 1897. Journ. Physiol., xxii, 215.
1900. Journ. Physiol., xxv, 417.
LANGLEY, J. N. AND H. K. ANDERSON. 1895. Journ. Physiol., xix, 122.
LIPSCHÜTZ, A. AND H. E. VON VOSS. 1924. Compt. rend. soc. biol., xc, 201.
MARCONI, P. 1923. Compt. rend. soc. biol., lccviii, 356.
REMY, C. 1886. Journ. de l'Anat. et Physiol., xxii, 205.
SHERRINGTON, C. S. 1892. Journ. Physiol., xiii, 685.
SPINA, A. 1897. Quoted by E. RETTERER in Richet's dictionnaire, v, 519.
TAKAHASHI, N. 1922a. Pflüger's Arch., cxcii, 322.
1922b. Pflüger's Arch., cxcvi, 237.

THE RESPIRATORY QUOTIENT OF PROTEINS IN PANCREATIC DIABETES

ELAINE P. RALLI,[1] ATTILIO CANZANELLI AND DAVID RAPPORT

From the Department of Physiology, Tufts College Medical School, Boston

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The present research was undertaken to discover whether, following the ingestion of relatively large amounts of proteins, the respiratory quotient of depancreatized dogs would indicate a failure of the organism to oxidize the glucose moiety of the proteins, and if so, incidentally to determine the amount of glucose deriving from the proteins.

THEORETICAL. Lusk (1915) has calculated that with a D/N ratio of 3.65/1, the R.Q. of meat protein would be 0.632; with a D/N ratio of 2.75/1, it would be 0.697. The Lusk ratio of 3.65/1 implies a yield of 59 grams of glucose from 100 grams of meat protein. Janney (1915) has estimated that gelatin yields 65 per cent glucose, and casein 48 per cent. Recently one of us (D.R., 1930, P. 398) has calculated that a theoretical yield of 44 per cent glucose could be obtained from meat protein, 58 per cent from gelatin, and 36 per cent from casein.

Extending this, we have attempted to calculate the theoretical R.Q. of these proteins in the completely diabetic state with a view to experimental test. The calculation in respect to meat protein is summarized in table 1.[2] Assuming a minimum molecular weight of 16,000 (according to Loewy's formula), and the present data for the amino acid constitution of meat protein, and knowing the molecular weights of the amino acids, the probable number of mols of each amino acid in the protein molecule can be arrived at, as well as the total molecular constitution of the sugar-forming and non sugar-forming amino acids. From the data obtained chiefly by Lusk and Dakin, we have compiled the carbon available for glucose in the protein, and thence the amount of glucose itself. Deducting urea, SO_3, and the theoretical yield of glucose, we have then calculated the theoretical R.Q., which by this method is for meat protein 0.73, the per cent yield of glucose being 43.9, and the D/N, 2.70/1. The extent of accuracy is checked in the first place by the normal R.Q. obtained with this method,

[1] Of the Department of Medicine, New York University and Bellevue Hospital Medical College.

[2] Tables 1, 2, and 3 have been abridged for publication. We shall be glad to furnish the full calculations to anyone interested.

which is 0.82 as compared with the classical 0.80 as calculated by Loewy, who took into account other nitrogenous excreta in addition to urea. In the second place the D/N ratio of 2.70 is reasonably close to the Minkowski ratio. This is the more striking in view of the fact that the amount of carbon available for glucose formation has been obtained largely from phlorhizinized dogs, in whom the D/N ratio is apt to approximate 3.65/1.

Similar calculations were made in respect to gelatin and casein. In the former, (table 2), a theoretical diabetic R.Q. of 0.65 was obtained, with a D/N of 3.21/1; in the latter (table 3), an R.Q. of 0.78, the D/N being 2.13/1.

TABLE 1

*Theoretical diabetic R.Q. of meat protein**

	MOLECULAR CONSTITUTION					CARBON AVAILABLE FOR GLUCOSE
	C	H	O	N	S	
Sugar-forming amino acids	359	718	238	118	6	237 (39 mols glucose)
Non-sugar-forming amino acids	284	524	90	62		
Total	643	1242	328	180	6	
Deducting urea (90 mols)	90	360	90	180		
Deducting 6 SO_3			18		6	
Normal R.Q† 0.82	553	882	220			
Deducting 39 mols glucose	234	468	234			
Residuum for respiratory exchange	319	414	−14			

Diabetic R.Q.† $= \frac{314\ CO_2}{429.5\ O_2} = 0.73$

* Using Loewy's formula.

† Deducting CO_2 corresponding to 10 mols of NH_3.

Experimentally, diabetic R.Q.s have as a rule been obtained from animals and human beings who were either fasting or on a protein-fat diet, rarely under such conditions that protein might be considered as the chief fuel. In depancreatized dogs, Hédon (1926) has obtained R.Q.s of 0.72 to 0.75 on administering 100 and 200 grams of meat. Macleod (1926, p. 126) records one experiment in which a depancreatized dog had an R.Q. of 0.71 after receiving 450 grams of meat on the 6th day after the last food and insulin, the preceding starvation quotients ranging from 0.66 to 0.72. We believe these results to be highly significant, as will be indicated later. Recently Chambers and Lusk (1930) on giving 300 grams of meat to a phlorhizinized dog, have obtained an R.Q. of 0.70 −0.71, the fasting R.Q.s being 0.69 and 0.70.

METHOD. Depancreatized dogs were used. The experiments were all performed from one to five months after pancreatectomy. During this

TABLE 2

*Theoretical diabetic R.Q. of gelatin**

	MOLECULAR CONSTITUTION				CARBON AVAILABLE FOR GLUCOSE
	C	H	O	N	
Sugar-forming amino acids	265	559	182	93	199 (33 mols glucose)
Non-sugar-forming amino acids	81	163	26	21	
Total	346	722	208	114	
Deducting urea (57 mols)	57	228	57	114	
Normal R.Q.† = 0.86	289	494	151		
Deducting 33 mols glucose	198	396	198		
Residuum for respiratory exchange	91	98	−47		

$$\text{Diabetic R.Q.}\dagger = \frac{90\ CO_2}{139\ O_2} = 0.65$$

* Min. Mol. Wt. = 10,300 (Cohn, E. J., J. T. Hendry and A. M. Prentiss. Journ. Biol. Chem., 1925, lxiii, 721).

† Deducting CO_2 corresponding to 2 mols NH_3.

TABLE 3

*Theoretical diabetic R.Q. of casein**

	MOLECULAR CONSTITUTION				CARBON AVAILABLE FOR GLUCOSE
	C	H	O	N	
Sugar-forming amino acids	233	436	170	58	146 (24 mols glucose)
Non-sugar-forming amino acids	233	434	76	48	
Total	466	870	246	106	
Deducting urea (53 mols)	53	212	53	106	
Normal R.Q.† = 0.86	413	658	193		
Deducting 24 mols glucose	144	288	144		
Residuum for respiratory exchange	269	370	49		

$$\text{Diabetic R.Q.}\dagger = \frac{263\ CO_2}{337\ O_2} = 0.78$$

* Min. Mol. Wt. 12,800 (Cohn, Hendry and Prentiss. Loc. cit.).

† Deducting CO_2 corresponding to 12 mols NH_3.

interval the animals were kept on a maintenance diet of beef heart, lard, raw pancreas and cracker meal (in some cases cane sugar was substituted

for the cracker meal). Insulin was administered in amounts not quite sufficient to keep the dogs sugar-free. Experiments were performed on the 3rd and 4th day after the last maintenance food and insulin. It was found difficult to continue experimentation on the same animal after the fourth day, on account of vomiting of the experimental diet and rapid deterioration of the animals. One dog is still alive at this writing. Autopsies on animals which died revealed no evidence of remaining pancreatic tissue.

On the experimental day 80 to 150 grams of protein in the form of either lean beef heart, gelatin or casein were given to the animal. Partly because, as we shall show later, we had reason to believe that the metabolism under these circumstances was almost exclusively protein, no attempt was made to differentiate between "protein" and "non-protein" R.Q.s, as is ordinarily done in experiments of this nature. In addition to this, such an attempt would have been begging the question, for in order to make such a differentiation, values for the protein R.Q. must be assumed, and the purpose of our work was to establish such values under the given conditions.

The respiratory exchange was obtained by a closed system of the Benedict-Homans type, of about 150 liters capacity. The blood sugar was determined before and after the experiment by the Folin-Wu method. In some instances so was also the blood CO_2 content (by the method of Van Slyke and Stadie), in order to see whether a change might introduce an appreciable artifact in the R.Q. As will be seen in ensuing tables, the changes observed were slight, the resulting maximum error from this source being negligible, and within the limits of the experimental eror. The blood CO_2 determination was therefore omitted in the later experiments. Urinary sugar was determined by the Benedict micro method.[3]

In some instances, the experiment following protein was preceded by a determination of the basal metabolism, in order to establish a starvation R.Q. on the same day. This precaution proved to be unnecessary, as the basal quotients were remarkably constant.

During the course of the experiments, the apparatus was tested by alcohol and acetone checks. Ten alcohol checks were performed, the average R.Q. being 0.658, the maximum error being 2.4 per cent. Five interspersed acetone checks showed an average R.Q. of 0.753 (as compared with the theoretical 0.750), the maximum error being 1.4 per cent. The maximum error of the apparatus was therefore no greater than 0.02 in the R.Q., the mean error being less than 0.01.

EXPERIMENTAL RESULTS. A. *Basal metabolism.* Eight determinations of the starving metabolism were made (at least two days after the last insulin and food). The results are summarized in table 4. The respir-

[3] We wish to express our indebtedness to Dr. M. G. Banus for his kindness in making the blood CO_2 determinations, and to Mr. J. Rosenman for some of the determinations of blood and urinary sugar.

atory quotients were very constant, and were uniformly above 0.70. This is not unusual in depancreatized animals, although not infrequently in the literature quotients as low as 0.68 are observed (see Rapport, 1930, p. 432). On the whole, the average starving R.Q. in depancreatized dogs has been somewhat lower than in normal dogs, and as we shall see later, this may in part be attributed to the lower quotient of the animal's endogenous pro-

TABLE 4

The basal metabolism

DOG NUMBER	DATE	DAYS AFTER LAST INSULIN	WEIGHT	URINARY N PER HOUR	RESPIRATORY EXCHANGE PER HOUR			REMARKS
					CO_2	O_2	R.Q.	
	1929		*kilos*	*gram*	*grams*	*grams*		
9	Dec. 13	4	9	0.280	7.35	7.68	0.696	Last starving D/N = 3.42/1
	1930							
13	June 19	2	8.1	0.249	5.93	6.10	0.707	Last starv. D/N = 5.02/1 Bl. Sugar = 323 mgm. %
13	June 20*	3	8.0	0.458	6.45	6.63	0.708	Bl. Sugar = 231 mgm. %
14	June 12	2	6.5	0.121	5.13	5.02	0.737	Last starv. D/N = 7.33/1 Bl. Sugar = 346 mgm. %
10	June 16	2	6.5	0.133	5.02	5.16	0.708	Last starv. D/N = 6.60/1 Bl. Sugar = 274 mgm. %
10	June 17	3	6.2	...	5.24	5.39	0.707	Bl. Sugar = 229 mgm. %
10	June 26	2	6.4	0.118	5.14	5.22	0.717	Last starv. D/N = 6.0/1 Bl. Sugar = 381 mgm. %
10	July 8	2	5.7	...	4.75	4.83	0.715	Last starv. D/N = 3.50/1 Bl. Sugar = 377 mgm. %
Average							0.71	

* Died the following day.

tein metabolism. The fact that basal quotients are so frequently above 0.70 we believe to be of significance in connection with the diabetic R.Q. of muscle protein, confirming a yield of about 44 per cent glucose. It is of interest that the pre-mortal rise of protein breakdown observed in dog 13 did not alter the R.Q.

The administration of meat protein. Meat protein was administered in the form of finely chopped lean beef heart, in amounts varying from 450 to

TABLE 5

The administration of meat protein

DOG NUMBER	DATE	DAYS AFTER LAST INSULIN	WEIGHT	AMOUNT‡ OF MEAT FED	URINARY N PER HOUR	RESPIRATORY EXCHANGE PER HOUR*			REMARKS
						CO_2	O_2	R.Q.	
	1930		*kilos*	*grams*	*gram*	*grams*	*grams*		
11	Jan. 8	2	5.0	450††	0.585	6.94	7.02	0.719	Last starving D/N = 3.01/1 Bl. Sugar Before = 278 mgm. % After = 304 mgm. % Bl. CO_2: Before = 42 vols. % After = 46 vols. %
11	Jan. 13	2	4.8	470††	0.620	7.26	7.27	0.726	Starv. D/N = 4.53/1 Bl. Sugar: Before = 349 mgm. % After = 435 mgm. % Bl. CO_2: Before = 37 vols. % After = 41 vols. %
13	Apr. 1	3	9.0	600††	0.765	10.06	10.86	0.674	Bl. Sugar: Before = 204 mgm. % After = 274 mgm. % Bl. CO_2: Before = 40 vols. % After = 42 vols. %
13	June 9	2	8.9	500††	†	8.61	8.66	0.723	5 hour period Bl. Sugar: Before = 345 mgm. % After = 257 mgm. %
13	June 19	2	8.7	600	0.737	9.52	9.61	0.721	Starv. D/N = 5.02/1 Bl. Sugar: Before = 323 mgm. % After = 274 mgm. %
10	June 16§	2	7.0	500**	†	7.29	7.44	0.713	3 hour period Starv. D/N 6.60/1 Bl. Sugar: Before 274 mgm. % After 279 mgm. %
Average								0.72	

* 4 hr. expt. unless otherwise noted.

† Not obtained. Dog voided.

‡ Given with 50 grams raw pig pancreas, unless otherwise noted.

§ 3 hr. expt.

** Given with 35 grams raw pig pancreas.

†† Given with 20 grams raw pig pancreas.

600 grams, together with a variable amount of raw pig pancreas freed as far as possible from fat and membranes. The pancreas was given to facilitate the digestion of the meat, and it was felt that the small quantity given would not appreciably affect the respiratory quotient. (This was borne out by the fact that omitting or altering the amount of pancreas had no influence on the ensuing R.Q.) Following the meat feeding, the animals were placed in the calorimeter, and after a preliminary period of an hour the determination of the respiratory exchange was carried out for a period usually of 4 hours. The results are summarized in table 5. With one exception, the quotients for the first few hours after giving meat to the depancreatized dogs were uniformly in the neighborhood of 0.72. (We are unable to account for the low R.Q. of dog 13 on April 1. On two other days, this dog's quotients were definitely higher.) The changes in blood CO_2 during the experiments, when measured, did not invite the presumption of artifact.

The question arises whether the animal's R.Q. under these circumstances can be said to be a function of the R.Q. of the administered protein. Judging from the N excretion of the experimental periods, it would appear that only about 43 to 51 per cent of the metabolism was due to protein. Even were this true, the ingested material would still have had an unmistakable effect upon the animals' R.Q., and if the sugar of this amount of protein had been oxidized a resulting R.Q. as high as 0.76 should have been obtained. It is well known, however, that after the ingestion of protein, the nitrogen excretion lags considerably behind the protein actually catabolized during a short period (4 to 5 hours), and previous experience (cf. Weiss and Rapport, 1924) suggested that under the conditions the metabolism was almost exclusively protein. We have tested this by three experiments on normal dogs. To one dog living on a "standard" diet, 400 grams of beef heart were administered. In spite of the fact that the protein metabolism, as judged by the nitrogen elimination during the five hour experimental period was only 40 per cent of the total, the R.Q. was 0.80, or theoretical for the normal combustion of protein. However, in this experiment it might be said that the non-protein quotient might also have been 0.80, and the result a coincidence. To another dog, therefore, whose basal R. Q. after 48 hours of starvation was 0.75, we gave 400 grams of beef heart. The N elimination indicated a 50 per cent protein metabolism, but the R.Q. was 0.79. After two more days of starvation, the basal R.Q. was 0.73 (non-protein 0.71), while after 500 grams of meat the R.Q. was again 0.79, proving that the metabolism was practically exclusively protein in character, in spite of the fact that according to the N elimination only 56 per cent of the energy exchange was due to protein.

We believe therefore that the quotients obtained in the diabetic animals may be accepted as legitimate expressions of the actual R.Q. of the beef

heart under the circumstances, with the possible qualification that a very small amount of fat may have been oxidized. If the results are compared with the theoretical diabetic R.Q. of meat protein, it will be seen that there is a very close agreement, for the theoretical quotient was 0.73 (it is of course admitted that the constitution of beef heart is not quite the same as the Loewy formula for muscle protein). The agreement, in fact, is well within the limit of error of both the theoretical calculation and the experiments themselves. This agreement implies a complete failure on the part of the dogs to oxidize sugar derivable from meat protein.

In addition, it is confirmatory of the Minkowski ratio for these animals, for the diabetic protein R.Q. of 0.73 is associated with a D/N of 2.70/1. As has been stated, the Lusk ratio of 3.65/1, which is thoroughly well established for completely phlorhizinized dogs, implies a 59 per cent yield of glucose and a quotient of 0.63. In calculating the ketogenic- antiketogenic ratio in human diabetics it is assumed that 58 per cent of the protein molecule may appear as sugar, and it must be admitted that in severe diabetics, D/N ratios as high as 4/1 have been obtained under trustworthy conditions. We believe that in the depancreatized dog, at least, the sugar obtainable from muscle protein is in the neighborhood of 44 per cent. Whether this applies to human diabetics is a moot question.

If our results and the Minkowski ratio are correct as regards the depancreatized dog, the high D/N ratios obtained by Macleod and his coworkers in the first few days after discontinuance of insulin (and in fat animals for longer periods) are due simply to delay in the depletion of glycogen reserves. In our own experiments, the D/N ratios on the second day after discontinuance of insulin have tended also to be high (see table 5), yet following this period, the experimental R.Q.s indicated no evidence of sugar oxidation or of the conversion of fat into sugar (the latter, with a high D/N, would have necessitated a lowering of the R.Q. below 0.70, the amount of lowering depending on the amount of the conversion).

D/N ratios of the experimental period itself were calculated in 3 experiments, the figures being 4.28, 4.23, and 5.42. The ratios over this short period are meaningless in respect to the sugar derivable from protein, as the sugar of ingested and metabolized protein is excreted more rapidly than the nitrogen.

The administration of gelatin. We experienced some difficulty in giving gelatin, as the animals were loath to eat it, and were apt to regurgitate after the feeding. However, we were able to obtain three satisfactory experiments, whch are summarized in table 6. In two of these experiments (on dog 13), in which the specific dynamic action and nitrogen excretion indicated a large protein metabolism, the average R.Q. was 0.68. In the third experiment the quotient was somewhat higher (0.70), and was associated with a smaller metabolism of the gelatin. We performed still an-

other experiment, in which the R.Q. following the feeding of gelatin was 0.72. But in this experiment it was clear that for some reason the gelatin was not being absorbed, for the nitrogen excretion was very little elevated above the starving level and there was practically no specific dynamic action, hence we have omitted it from the table of satisfactory experiments.

In the 3 experiments the average quotient was 0.69. Reverting to table 2, it will be seen that our theoretical diabetic quotient for gelatin was 0.65. While the agreement is not perfect, the R.Q. of the experiments was

TABLE 6

The administration of gelatin

DOG NUMBER	DATE	DAYS AFTER LAST INSULIN	WEIGHT	AMOUNT OF PROTEIN FED	URINARY N PER HOUR	RESPIRATORY EXCHANGE PER HOUR			REMARKS
						CO_2	O_2	R.Q.	
	1930		*kilos*	*grams*	*gram*	*grams*	*grams*		
13	Jan. 23*	2	7.6	100	0.794	9.61	10.11	0.691	Starv. D/N = 2.28/1‡ Bl. Sugar: Before = 270 mgm. % After = 286 mgm. % Bl. CO_2: Before = 44 vols. % After = 43 vols. %
13	June 20†	3	8.0	100	0.947	8.87	9.61	0.671	Starv. D/N = 5.02/1 Bl. Sugar: Before = 231 mgm. % After = 272 mgm. %
14	Jan. 27§	2	6.5	75	0.509	7.83	7.97	0.704	
Average								0.69	

* 4 hr. expt.

† 2 hr. expt. Dog vomited during 3rd hour.

‡ 6 hr. specimen.

§ 3 hr. expt.

definitely lower than in both the starving state and after the administration of meat, as one would expect from the theory. Our experimental results would indicate that about 53 per cent of the gelatin molecule is convertible to glucose, as compared with 58 per cent in the theoretical calculation.

The administration of casein. In the case of this protein, there was a definite discrepancy between the theory, which indicated a diabetic R.Q. of 0.78, and our experimental results. In five experiments, using two differ-

ent brands of casein (Harris, and Eimer and Amend), the resulting respiratory quotients were consistently in the neighborhood of 0.70 (table 7). This discrepancy is considerably beyond our margin of error, and we are unable to explain it, unless the caseins employed had a different composition than the one upon which the theoretical calculation was based. The

TABLE 7

The administration of casein

DOG NUMBER	DATE	DAYS AFTER LAST INSULIN	WEIGHT	AMOUNT OF PROTEIN FED	URINARY N PER HOUR	RESPIRATORY EXCHANGE PER HOUR			REMARKS
						CO_2	O_2	R.Q.	
	1930		*kilos*	*grams*	*gram*	*grams*	*grams*		
11	Jan. 14†	3	5.0	100*	0.590	6.06	6.23	0.712	Starving D/N = 4.53/1 Bl. Sugar: Before = 286 mgm. % After = 250 mgm. %
13	Mar. 31†	2	8.7	100*	0.785	8.45	8.76	0.702	Bl. Sugar: Before = 305 mgm. % After = 271 mgm. % Bl. CO_2: Before = 44 vols. % After = 46 vols. %
13	June 10‡	3	8.9	105	...	7.86	8.34	0.685	Bl. Sugar: Before = 266 mgm. % After = 244 mgm. %
14	Jan. 28‡	3	6.5	80*	0.570	7.92	8.19	0.704	Bl. Sugar: Before = 290 mgm. % After = 286 mgm. %
10	June 17‡	3	6.6	80	0.455	6.44	6.64	0.704	
Average								0.70	

* Given with 50 grams raw pig pancreas.

† 5 hr. expt.

‡ 3 hr. expt.

quotients actually obtained imply a yield of about 50 per cent glucose from the casein molecule, and indicate that this glucose is not oxidized, for if it were, the resulting R.Q. would have approached the normal quotient of the protein, namely, 0.84 to 0.86.

It is of interest that in these experiments, as well as in the case of the other two proteins, the ingestion of the foodstuff did not cause any appre-

ciable increase in the blood sugar at the end of approximately 5 hours after this feeding. In fact—and this was particularly true of casein—there was often manifested a fall.

Attempts to give large amounts of glucose by mouth were unsuccessful, as the material was vomited, and no satisfactory experiment was obtained.

Discussion. When fat is administered to a diabetic animal, the resulting respiratory quotient is in the neighborhood of 0.70 (unless modified by the artifact of acidosis). This might be due to the direct oxidation of fat. If it be assumed, however, that the fat has been converted to carbohydrate, then the latter must have been immediately oxidized, else the R.Q. would have tended to approach the quotient of the conversion, which is about 0.3. Therefore, the assumption of the conversion leads to the theory that in diabetes there is no failure of the sugar oxidative mechanism, but rather an overproduction of sugar from protein and fat.

When, in a complete diabetic, the energy exchange is largely due to the combustion (directly or indirectly) of fat, as under the conditions of starvation or of ordinary diet, the observed quotients of 0.68 to 0.72 may conceivably be interpreted as due to overproduction of sugar from fat, and to a lesser extent from protein, with oxidation of at least the major portion of this sugar. But in the experiments here reported, the metabolism was chiefly, and probably entirely, due to protein, and the animals' R.Q. was therefore practically the R.Q. of the protein. Calculating a theoretical respiratory quotient from meat protein on the basis that none of the sugar deriving from it was oxidized, we find that the actual R.Q. obtained was almost identical with it. If the sugar had been oxidized, the quotient would have tended to approach 0.80, the normal quotient for meat.

Since there is no reason to believe that sugar obtained from protein would behave differently from sugar derivable from fat, then if the former is not oxidized, neither is the latter. But as we have already stated, the R.Q. of fat-fed diabetics indicates that if fatty acids be converted to sugar, the latter must be oxidized. Admitting the correctness of the above reasoning, it follows that fatty acids are not converted to carbohydrate under the conditions. If they are not, then the primary defect in pancreatic diabetes is a failure of the sugar oxidative mechanism rather than an overproduction of sugar. Only a few physiologists at present believe the latter, but many believe in the convertibility of fatty acids to glucose. Yet one would appear to be directly related to the other.

SUMMARY

1. According to a theoretical calculation, based on the assumption that the sugar deriving from protein was not oxidized, the respiratory quotient of muscle protein in complete diabetes should be 0.73, of gelatin 0.65, and of casein 0.78.

2. In depancreatized dogs, given sufficient protein so that it could be assumed that the R.Q. of the animal was a function of the R.Q. of the administered protein, we have found, after meat, a quotient of 0.72, the average starvation R.Q. being 0.71.

3. We regard this concordance as evidence validating the Minkowski ratio, and as opposed to *1*, the theory that in pancreatic diabetes the primary defect is not the failure of the sugar oxidizing mechanism; *2*, the theory that fatty acids are convertible to sugar.

4. After administering gelatin, the average respiratory quotient was 0.69, which we regard as reasonably confirmatory of our calculation for this protein. After casein, however, the quotient was consistently lower (0.70) than was expected from the theory. We are unable to explain this discrepancy.

BIBLIOGRAPHY

CHAMBERS, W. H. AND G. LUSK. 1930 Journ. Biol. Chem., lxxxv, 611.
CHAIKOFF, I. L. AND J. J. R. MACLEOD. 1929. Quart. Journ. Exp. Physiol., xix, 291.
HÉDON L. 1926. Arch. Intern. Physiol., xxvii, 354.
JANNEY, N. W. 1915. Journ. Biol. Chem., xx, 321.
LUSK, G. 1915. Arch. Int. Med., xv, 939.
MACLEOD, J. J. R. Carbohydrate metabolism and insulin. New York, 1926.
RAPPORT, D. 1930. Physiol. Rev., x, 349.
WEISS, R. AND D. RAPPORT. 1924. Journ. Biol. Chem., lx, 513.

CONTINUOUS PANCREATIC SECRETION IN THE RABBIT

STEWART G. BAXTER

From the Department of Physiology, McGill University, Montreal, Canada

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Continuous pancreatic secretion in the rabbit is a well established fact. It was observed by Heidenhain (1877) in rabbits anesthetized with curare, by Pavlov (1878) in unanesthetized rabbits, by Gottlieb (1894) in rabbits under urethane, and by Savitsch (1918) in spinal rabbits. After the intravenous injection of pilocarpine Gottlieb reported an increase in the amount of secretion and also in the total solids of the juice. On the other hand, Savitsch found that stimulation of the vagi in the neck had no effect on the pancreatic secretion in the rabbit.

The continuous pancreatic secretion in the rabbit was attributed by Terroine (1913) to the unceasing digestive work of the stomach and hence to the continuous action of the pancreatic hormonal mechanism. The part played by the parasympathetic and sympathetic innervation in the pancreatic secretion was not at all clear. An attempt has been made in the present investigation to determine how far the continuous pancreatic secretion is controlled by humoral influences. The nervous control of the pancreatic secretion in the rabbit is dealt with in another paper (this issue).

METHODS. Adult rabbits were used in all our experiments. Certain difficulties were encountered in the choice of anesthetics, which it is well to point out, as they have an important bearing on the interpretation of the experimental results. Anesthetics that were injected intraperitoneally, such as dial and amytal (isoamyl ethyl barbituric acid), produced a marked vaso-dilatation of the abdominal viscera and a sero-hemorrhagic exudate in the peritoneal cavity. As a result of this irritation an abnormally copious flow of pancreatic juice was produced. Sodium luminal injected intravenously in doses sufficient for anesthesia caused a serious depression of circulation and respiration, and the animals frequently died from cardio-respiratory failure two or three hours after anesthetization. Chloralose gave good anesthesia, but it activates the secretion of adrenaline (Vincent and Thompson, 1928; MacKay, 1929) which is greatly antagonistic to pancreatic secretion, causing vaso-constriction of the pancreatic blood vessels. Urethane injected intravenously (1 gram per kgm. of a 20 per cent solution in normal saline) was found to give the most satisfactory anesthesia. Experiments were also done on decerebrate and decapitate animals.

The pancreatic duct was cannulated with a small metal cannula and in experiments where a kymographic record of secretion was taken, the cannula was connected by means of fine rubber tubing to a Gibbs drop recorder. In other experiments the

juice was collected with a fine glass pipette and kept in hourly samples. In this way an accurate correlation of the rate of secretion and the enzyme content of the juice could be made. In all the experiments the pylorus and bile duct were ligated in order to exclude the uncontrolled flow of gastric and biliary secretions into the duodenum.

To determine the proteolytic ferment the milk coagulation method developed by Mellanby (1912) was used. The enterokinase solution for activation of protrypsin was prepared by a uniform method, as follows: 30 grams of intestinal mucosa were ground with 100 cc. of distilled water, and, after standing overnight, this was filtered and a few cubic centimeters of toluene added. This solution, if kept in a cool place, retained its activity for several weeks. New solutions were always checked against the old for potency. The time of coagulation of the milk solution in seconds was converted into an arbitrary value in units having an inverse relationship to the time of coagulation. A series of duplicate determinations showed that the experimental error was less than 10 per cent. This method, which permitted of frequent determinations during the course of an experiment, was used as an index of enzymatic power in most of the experiments.

For determining the amylolytic power of the juice the method of Waksman (1920) was found satisfactory.

To measure the lipolytic power of the juice the method of Mellanby (1925) was adopted. The determinations of the lipolytic power of the pancreatic juice were always made as soon as the specimens had been collected, as auto-digestion of this ferment may take place although enterokinase has not been added (Mellanby and Woolley, 1918).

Since all three enzymes are secreted in parallel concentration (Babkin, 1904; Savitsch, 1909), we determined only the proteolytic ferment in the majority of the experiments. A certain number of experiments were done to determine whether the pancreatic secretion in the rabbit adhered to the general rule of parallel secretion of enzymes, and this was found to be the case.

EXPERIMENTAL DATA. After the discovery of the stimulating effect of acid on the pancreatic secretion and later of the humoral mechanism of its action, it was assumed that the continuous pancreatic secretion in the rabbit noted by all investigators was due to the constant presence of acid chyme in the duodenum and the consequent liberation of secretin into the blood (Terroine, 1913). It was observed in the course of the present investigation that injection into the duodenum of 0.2 per cent HCl, as well as of a mixture of HCl, bile and peptone, greatly increased the continuous pancreatic secretion, but at the same time a marked fall in the enzyme power was noted.

In our experiments continuous pancreatic secretion was observed for as long as 10 to 12 hours in rabbits anesthetized with urethane. In decerebrate or decapitate animals it continued for 5 to 6 hours, until the death of the animal. Our next step was to consider the effect of removing the constant humoral stimulus. This could be most simply accomplished by ligature of the pylorus and common bile duct. Experiments carried out under this technique showed that the secretion continued unabated and without any marked change in enzyme content for 10 to 12 hours.

Since it did not seem logical that the small amount of chyme remaining

in the small intestine could activate the gland for 10 to 12 hours after ligature of the pylorus, it was decided to observe the effect on the continuous secretion of removal of the stomach and the small intestine, and finally of complete removal of the gastro-intestinal tract (from the lower end of the esophagus to the lower end of the rectum), the pancreatic gland being left intact. To accomplish this without unduly damaging the pancreatic gland, which is very delicate, and without interfering with the blood supply, required special operative care. The pancreatic gland in the rabbit receives its blood supply from three main sources: 1, the upper part of the body from the superior pancreatico-duodenal artery which is a branch of the gastro-duodenal artery; 2, the lower two-thirds of the body from the inferior pancreatico-duodenal artery which is a branch of the superior mesenteric artery; and 3, short branches from the splenic artery supplying

TABLE 1

Experiment May 19

Rabbit 1.8 kgm. Urethane 1 gram per kgm. I.V. Complete extirpation of gastro-intestinal tract.

TIME		SECRETION	
		Volume	Trypsin
		cc.	units
10–11	During operation	0.35	240
11:30	Evisceration complete		
11:30–12:30		0.2	220
12:30– 1:30		0.2	225
1:30– 2:30		0.15	480
2:30– 3:30		0.05	480

the tail of the pancreas. Now, by tying the duodenal branches of the superior and inferior pancreatico-duodenal arteries close to the duodenum, it was possible to separate the gland from the duodenal loop to which it was attached, without interfering unduly with the blood supply. A small section of duodenum containing the opening of the pancreatic duct was left intact as a support for the duct and cannula.

The removal of the stomach required a careful study of the relationship of the gastric and pancreatic blood supplies. Finally a technique was developed for removing the stomach without impairment of the pancreatic blood supply. The splenic branches to the stomach were tied close to that organ, thus allowing the short branches given off to the pancreas to remain undisturbed. The esophagus was then divided and the numerous branches of the left gastric and hepatic arteries were tied close to the stomach. Following along the lesser curvature the gastric and gastro-epiploic vessels were ligated, care being taken to avoid the superior pancreatico-duodenal

branch of the gastro-duodenal artery. The bile duct was tied and cut. The pancreas was then separated from the upper part of the duodenum and the first inch or two of the duodenum tied and divided. By ligating any remaining vessels in the omentum the stomach could easily be removed without any serious loss of blood. After the extirpation of the stomach the small gut and large intestine were removed.

Table 1 shows an experiment in which the gastro-intestinal tract was completely removed, the pancreatic gland, with blood supply still intact, alone remaining in the abdomen. Analogous results were given by removal of the small intestine alone, and also of the stomach and small intestine.

TABLE 2

Experiment June 16

Rabbit 2.4 kgm. Ether. Decapitated. Complete extirpation of gastro-intestinal tract.

TIME		SECRETION	
		Volume	Trypsin
		cc.	*units*
11–12	During operation	0.4	400
12	Evisceration complete		
12–1		0.25	250
1–2		0.3	300
2–3		0.3	300
3–4		0.2	400
4–5		0.1	514

Before drawing any conclusions from these results it was considered necessary to control further the possible influence of anesthetics on the production of the pancreatic secretion in eviscerated animals. Consequently a number of experiments were performed on decapitate animals. After decapitation the artificial respiration was adjusted, and the animals were warmed and left for half an hour to allow them to recover from the shock and to eliminate the ether from the lungs.

The experiment (table 2) illustrates the effect of evisceration on the continuous secretion in a decapitate rabbit.

In this and similar experiments the blood pressure fluctuated between 30 and 40 mm. Hg. The enzyme concentration of the juice was usually normal, although in some of the experiments a fall in the enzyme content was noted. This was probably due to damage of the sympathetic nerve supply to the pancreas during removal of the gastro-intestinal tract.[1]

[1] The effect of removal of the sympathetic nerve supply to the pancreatic gland is discussed in the paper on "Nervous control of the pancreatic secretion in the rabbit."

From the results of the above experiments it may be concluded that the continuous secretion in the rabbit persists after extirpation of the whole gastro-intestinal tract in decapitate animals, as well as in animals anesthetized with urethane.

Discussion. The fact of continuous secretion was well known to previous investigators, but little or nothing had been established with regard to its causation. It was assumed *a priori* that the secretion was due to a continuous humoral stimulus. In contradiction of this supposition our experiments show that the secretion continues for 5 to 6 hours in decapitate animals in which the whole gastro-intestinal tract has been removed.

The significance of this result in relation to the secretin mechanism is not clear. Either the secretin mechanism of the rabbit differs fundamentally from that of other animals and the small amount of secretin circulating in the blood at the time of evisceration is capable of activating the secretion for long periods (which is extremely unlikely), or the basic cellular physiology of the pancreatic gland of the rabbit differs radically from that of carnivorous animals. In other words, according to the latter supposition, the secretory cells of the pancreatic gland of the rabbit are permeable at all times to water and salts which pass through them from the blood and carry out organic material and enzymes that have been stored in the cells.

Thus it appears that in the rabbit there is a true "spontaneous" secretion of pancreatic juice, which may be increased by exogenous stimuli, such as acid, bile and peptone, present in the duodenum and small intestine.

SUMMARY

1. Continuous pancreatic secretion in the rabbit may be traced in animals anesthetized with urethane for as long as 10 to 12 hours, and in decerebrate or decapitate animals for from 5 to 6 hours until the animal succumbs.

2. The pancreatic secretion in the rabbit adheres to the general rule of parallel secretion of enzymes.

3. The continuous pancreatic secretion persists unabated and without any marked change in enzyme content for 10 to 12 hours after ligation of the pylorus and the common bile duct. It likewise continues practically unchanged for 4 to 5 hours after evisceration (stomach and small and large intestines) in decapitate animals, as well as in animals anesthetized with urethane.

4. It is assumed that this continuous secretion is essentially a true "spontaneous" pancreatic secretion due to the particular properties of the pancreatic gland in the rabbit.

The writer wishes to express his indebtedness to Dr. B. P. Babkin, in whose laboratory this investigation was carried out, for much helpful advice and encouragement during the course of the work.

BIBLIOGRAPHY

BABKIN, B. P. 1904. Nachr. d. Kaiserl. Militar. Med. Akad., xlix, 93.
GOTTLIEB, R. 1894. Arch. f. exper. Path. u. Pharm., xxxiii, 265.
HEIDENHAIN, R. 1877. Pflüger's Arch., xiv, 457.
MACKAY, M. E. 1929. Journ. Pharm. Exper. Therap., xxxvii, 349.
MELLANBY, J. 1912. Journ. Physiol., xlv, 345.
1925. Ibid., lx, 85.
MELLANBY, J. AND V. J. WOOLLEY. 1918. Ibid., xlviii, 287.
PAVLOV, I. P. 1878. Pflüger's Arch., xvii, 555.
SAVITSCH, W. W. 1909. Zentralbl. f. d. ges. Physiol., no. 1.
1918. Journ. Physiol. Russe, i. 134.
TERROINE, E. F. 1913. La Sécrétion Pancréatique. Paris.
VINCENT, S. AND J. H. THOMPSON. 1928. Journ. Physiol., lxv, 449.
WAKSMAN, S. A. 1920. Journ. Amer. Chem. Soc., xlii, 293.

NERVOUS CONTROL OF THE PANCREATIC SECRETION IN THE RABBIT

STEWART G. BAXTER

From the Department of Physiology, McGill University, Montreal, Canada

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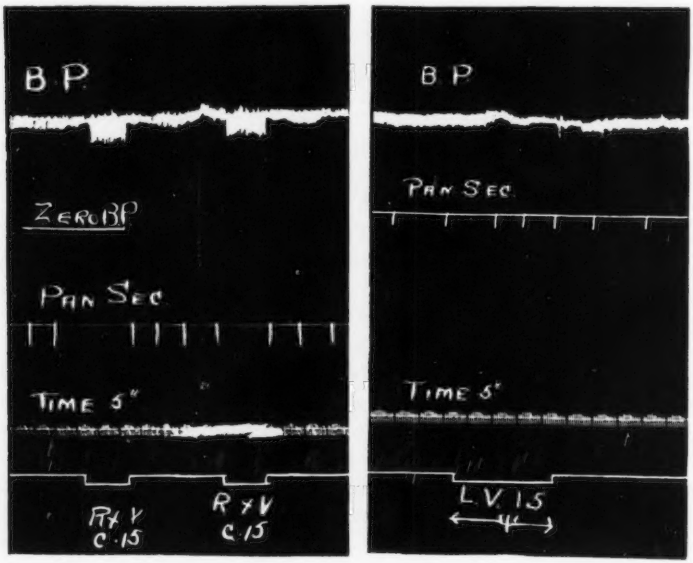
Physiological data concerning the parasympathetic innervation of the pancreatic gland in the rabbit are few, and there are none concerning the sympathetic innervation. Gottlieb (1894) found that pilocarpine injected intravenously caused an increase in the rate of secretion as well as in the amount of total solids in the pancreatic juice of the rabbit. According to him the continuous secretion showed certain irregularities, which he thought were caused by rhythmic contractions of the pancreatic ducts. Savitsch (1918) stimulated the vagi in the neck of a rabbit with the spinal cord sectioned below the medulla, but did not observe any increase in the amount of secretion. He concluded from his experiments that the vagus had no influence on the pancreatic secretion in the rabbit, and offered this fact as evidence in favour of the dualistic theory of pancreatic secretion. According to Savitsch, the pancreatic secretion in carnivorous animals, such as the dog, is regulated by nervous and humoral mechanisms, while in the rabbit only the humoral mechanism is necessary for the secretory activity of this organ.

In the present investigation the same methods of anesthetization, operation and determination of enzymes were used as in the experiments described in a previous communication (this issue). In most cases the rabbits were anesthetized by intravenous injection of urethane (1 gram per kilogram weight). In some of the experiments, to prevent any damage to the vagi by tying of the pylorus, a longitudinal incision was made through all the layers of the pylorus, and the mucous and submucous membranes were sewn by circular suture and tied.

Effect of stimulation of the vagi. In the course of numerous experiments the vagi were stimulated in the neck by means of rhythmic stimulation, or in the chest (below the heart) or in the abdomen (below the diaphragm) by means of an induction current. Stimulation in any of these sites resulted in a definite augmentation of the pancreatic secretion. In some of the experiments a kymographic record was taken; in another set of experiments the juice was collected directly from the cannula in hourly

or half-hourly portions. In the kymographic tracing a short phase of inhibition was usually noted, which in accordance with Anrep's experiments (1914) was probably due to a constriction of the pancreatic ducts. The inhibitory phase was followed in many cases by an acceleration of secretion (see fig. 1, A and B). When the secretion was collected directly, it was found that the amount increased to twice, or even three or four times, that of the control period. The enzyme content of the secretion increased



A B

Fig. 1. A. Rhythmic stimulation of the vagi in the neck, coil at 15 cm. Shows inhibition of secretion following stimulation.

B. Stimulation of the vagi in the chest below the heart with an induction current. Illustrates acceleration of secretion following a four-minute period of stimulation.

enormously after stimulation of the vagi, rising to three or in some cases to four times the control value. This result was observed consistently and is well illustrated in table 1.

It is important to note that in this experiment, as well as in all the others in which the vagi were stimulated, the digestive power of the juice secreted during stimulation of the parasympathetic nerve increased in spite of the dilution of the juice on account of its greater volume. In other words, we have here a "trophic" effect of the vagus, to use the term as it was employed by Heidenhain.

The results of these experiments indicate that the vagus has the same function in the rabbit as in the cat and dog, i.e., it contains secretory, trophic and motor inhibitory fibres to the ducts. Thus while the secretory function of the pancreatic gland of the rabbit must differ in some way from that of carnivorous animals, yet the essential effect of the parasympathetic innervation is analogous in both species.

The parasympathetico-mimetic drugs, pilocarpine and acetyl choline, have an effect similar to that of electrical stimulation of the vagus, since they also have the effect of augmenting the secretion and increasing the

TABLE 1

Experiment January 31. Rabbit 1.3 kgm. Urethane 1 gram per kgm. I.V. Bile duct tied. Mucous and submucous membranes of the pylorus sewn with a circular suture. Artificial respiration.

	TIME							
	10:15 to 11:15	11:15 to 12:15	12:15 to 1:30	1:30 to 2:45	2:45 to 3:45	3:45 to 4:45	4:45 to 5:45	5:45 to 6:45
Volume (cc.)	0.2	0.35*	0.1	0.2	0.1	0.1	0.3†	0.15
Trypsin (units)	600	1800	1440	800	514	514	1200	650

* Stimulation of alternate vagi in chest: 5 minutes stimulation and 5 minutes rest alternately for period of 1 hour. (Coil 10 cm.)

† Stimulation of alternate vagi in chest: 5 minutes stimulation and 5 minutes rest alternately for period of 1 hour. (Coil 9 cm.)

TABLE 2

Experiment June 5. Rabbit 1.2 kgm. Urethane 1 gram per kgm. I.V. Pylorus and bile duct patent. 11 a.m.—vagi cut below diaphragm.

	TIME							
	9–10	10–11	11–12	12–1	1–2	2–3	3–4	4–5
Volume (cc.)	0.6	0.6	0.5	0.6	0.6	0.3	0.7	0.7
Trypsin (units)	200	200	250	300	266	200	200	200
Lipase (cc. N/100 NaOH)	3.4	3.2	3.4	3.2	3.7		3.0	2.9

enzyme content of the juice. Thus in a certain experiment the intravenous injection of $\frac{1}{10}$ mgm. acetyl choline increased the hourly volume of the secretion from 0.2 to 0.35 cc. and the tryptic power from 900 to 1500 units. After a two-hour interval 1 mgm. of pilocarpine was injected intravenously and again there was an increase in the hourly volume (from 0.5 to 0.65) and also in the tryptic power (from 350 to 750 units).

EFFECT OF SECTION OF THE VAGI. A series of experiments was carried out to determine the result of removal of the central impulses conveyed through the vagi. For this purpose these nerves were cut either in the

neck or below the diaphragm. Mellanby (1925) has reported a sharp decline in the enzyme power of the pancreatic juice in a cat anesthetized with urethane and subjected to continuous injection of purified secretin solution. The same effect was observed after administration of atropine intravenously. Mellanby considered these results to be due to the removal of the constant stream of central impulses which ordinarily reach the pancreatic gland by way of the vagi.

The normal continuous pancreatic secretion of the rabbit offered a good medium for this type of experiment. In all our experiments involving

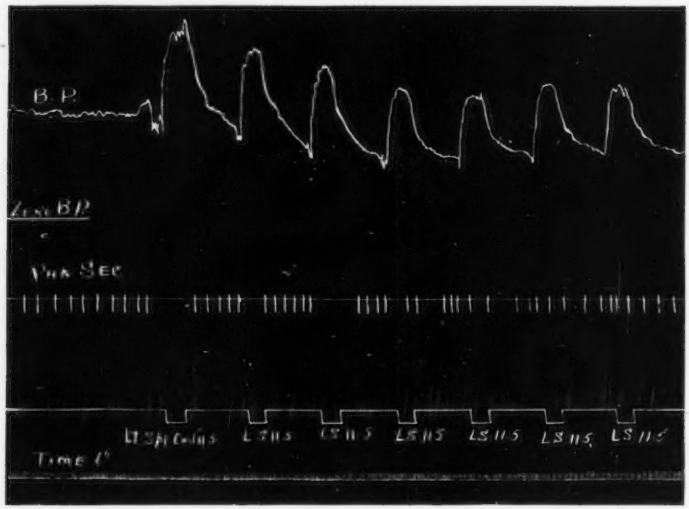


Fig. 2. Excitation of the left splanchnic nerve, adrenal vein not tied. Pylorus and bile duct ligated. Demonstrates initial vascular inhibition, which is followed by a pure secretory effect.

section of the vagi, the bile duct and pylorus were left patent, as it was desired to ascertain the effect on the normal secretion of removal of central vagal influences. A representative experiment of this type is shown in table 2.

Thus in the rabbit the removal, by section of the vagi above or below the diaphragm, of the central impulses which travel by way of the vagi to the pancreatic gland, has no marked influence on the rate of secretion or the enzyme content of the juice.

EFFECT OF STIMULATION OF THE SYMATHETIC NERVES. The sympathetic nerve supply to the pancreas comes with the blood vessels by plexiform

off-sets of the hepatic splenic and superior mesenteric plexuses which are relays of the coeliac plexus formed by the splanchnic nerves. Electrical stimulation of the splanchnic nerves is complicated by the fact that the sympathetic system supplies the pancreas with vaso-constrictor fibres and that the pancreatic gland is very sensitive to vaso-constriction. However, the problem is not so acute in the rabbit as in carnivorous experimental animals by reason of the continuous secretion in the former.

We stimulated the left splanchnic nerve in the abdomen of the rabbit, an incision being made in the left flank, so that it could be stimulated with as little disturbance of the viscera as possible and without any movement of the cannula, which was connected to a Gibbs drop recorder and a signal marker on the kymograph. In this way a kymographic record of the secretion was obtained, as well as samples of juice for ferment determinations. In some of the experiments the adrenal vein was tied; in others it was left untied. The typical effect of stimulation of the splanchnic nerve is seen in figure 2. From the figure it may be observed that the first three stimulations produced a typical inhibition of the secretion, which was probably due to vasoconstriction of the pancreatic vessels. During the next three periods of stimulation the inhibitory effect was not so marked, as one or two drops of secretion were recorded. On the 7th stimulation there was a definite increase in the secretion during the excitation of the nerve and no preliminary inhibition. The juice collected showed that the enzyme power had risen from 150 before to 350 after stimulation.

Stimulation of the splanchnic nerve with the adrenal vein tied gave better results. The rise in the blood pressure in this type of experiment was insignificant as compared with that in the previous type. The secretory effect of the splanchnic nerve was well shown in these experiments, since the acceleration occurred earlier in the stimulation and without any inhibition, and became more pronounced with each succeeding stimulation. The enzyme content of the juice also showed a definite increase after stimulation.

The increase in enzymes after stimulation of the splanchnic nerve cannot be attributed to concentration of the juice as the rate of secretion undergoes little, if any, diminution or may be increased and the ferment power amounts to two or even three times the control value. The initial inhibition followed by augmentation of the secretion during stimulation is probably due to the well known delicacy of the vaso-constrictor fibres under electrical stimulation. They rapidly become fatigued and the more resistant secretory fibres may then exert their true effect unconditioned by vascular inhibition.

Repeated injections of $\frac{1}{2}$ cc. of 1/10,000 solution of adrenaline gave the same result as electrical stimulation of the splanchnic nerve.

EFFECT OF SECTION OF THE SPLANCHNIC NERVES. A series of experi-

ments was undertaken in order to ascertain the effect of removal of the central impulses by section of both splanchnic nerves. The abdomen was opened and the splanchnic nerves identified, then gently freed and isolated on strong threads. A cannula was inserted and the incision closed. In this way, after the usual control period, the splanchnic nerves could be torn in two without re-opening the abdomen or disturbing the cannula. As before, the pylorus and bile duct were left open, since we wished to demonstrate the effect of the central impulses on the normal pancreatic secretion.

In all the experiments the section of the splanchnics was followed by an immediate sharp fall in the enzyme power of the secretion, which remained low. This effect may be seen in the following example from a series of analogous experiments (table 3).

After section of the splanchnic nerves the secretion increased temporarily to double the previous rate, and the enzyme content fell to one-quarter the previous figure. Subsequently the enzyme value fell still further. It

TABLE 3

Experiment June 27. Rabbit 2.2 kgm. Urethane 1 gram per kgm. I.V. Bile duct and pylorus patent. Both splanchnics isolated on threads; 12 noon—splanchnics torn in two.

	TIME							
	10–11	11–12	12–1	1–2	2–3	3–4	4–5	5–6
Volume (cc.)	0.6	0.6	1.1	0.9	0.2	0.6	0.5	0.7
Trypsin (units)	300	300	78	66	54	48	70	70

remained low for six hours, even when the secretory rate had returned to its former level. The abrupt fall in the ferment power could not therefore have been due to the transitory increase in the secretory rate caused by the section of the splanchnics and resultant lowered blood pressure and dilatation of the visceral blood vessels.

Accordingly it appears that in the rabbit the sympathetic nervous system in some way controls the enzyme content of the pancreatic secretion. The exact nature of this influence is not yet clear and requires further investigation.

SUMMARY

1. The vagus nerve, contrary to previous belief, is a true secretory nerve to the pancreatic gland in the rabbit, and possesses secretory "trophic" and motor fibres to the ducts.

2. The pancreatic secretion in the rabbit is stimulated by the parasympathetico-mimetic drugs, pilocarpine and acetyl choline.

3. Section of the parasympathetic nerves to the pancreatic gland has no marked effect on the rate of secretion or the enzyme content.

4. Stimulation of the splanchnic nerves in the rabbit produces, after a preliminary inhibition, an increase in the rate of secretion and a definite rise in the tryptic power of the pancreatic juice.

5. The repeated injection of small amounts of adrenaline produces the same results in the pancreatic secretion of the rabbit as stimulation of the splanchnic nerves.

6. Section of the sympathetic nerves in the rabbit produces an immediate and profound fall in the enzyme content of the pancreatic secretion, which has a lasting effect.

This investigation was carried out in the laboratory of Dr. B. P. Babkin, to whom the writer is indebted for much helpful advice and encouragement.

BIBLIOGRAPHY

ANREP, G. V. 1914. Journ. Physiol., xlix, 1.
GOTTLIEB, R. 1894. Arch. f. exper. Path. u. Pharm., xxxiii, 265.
MELLANBY, J. 1925. Journ. Physiol., lx, 85.
SAVITSCH, W. W. 1918. Journ. Physiol. Russe, i, 134.

DIURESIS BY CONDITIONED REFLEX

HELLMUT MARX[1]

From the Division of Medicine, The Mayo Clinic, Rochester, Minn.

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Recent investigations have shown that an exceedingly complicated mechanism in which various factors and different organs, including the brain and the central nervous system, are involved, is concerned in the regulation of the metabolism of water in the organism.

The experiments of Jungmann and Meyer, Bourquin, Camus and Roussy, Bailey and Bremer, Curtis, Verney, and others, indicate that a lesion of certain parts of the brain produces marked elimination of water and salt. The pathogenesis of diabetes insipidus shows clearly the importance of the central nervous system. Not only the midbrain but also the cortex has an influence on the metabolism of water, as was pointed out experimentally by von Bechterew and Ucko. It is probable, therefore, that psychic factors also influence the metabolism of water. In 1926, I pointed out that the suggestion of drinking produces distinct diuresis in hypnotized patients. These experiments have been confirmed by Hoff and Wermer and Bauer. It is also recognized that psychic factors, such as fear, excitement, and resistance to restraint, will influence the course of diuresis in animals.

In the experiments reported here an attempt was made to produce diuresis as a result of psychic factors by means of the establishment of the appropriate type of conditioned reflex.

These experiments constitute a part of a general study of the metabolism of water which has been carried out during the last year. Four dogs of approximately the same size and weight, but of different breed and temperament, were used for this metabolic study. Throughout the period of observation they were maintained on a steady diet containing dog biscuits and lard with a constant intake of fluid, given at the same time each day; a mixture of milk and water in the proportion of 1:2 was given as fluid. The body weight and the balance of water, nitrogen and sodium chloride in the urine were determined daily. A comprehensive analysis of the blood was made at intervals of one week. After the dogs had been accustomed to the experimental routine, and an accurate water balance had been established for each, it was decided to use them for the study of

[1] Special student in Medicine, The Mayo Foundation.

conditioned reflexes as well, even though it was recognized that they had not been selected primarily for this purpose.

The dogs were catheterized each morning at 8:15 with the exception of Sunday. They were then put in a special room where each was tied in its own place. After they became quiet, with each dog lying quietly, a musical signal was given with a small mouth organ. Directly thereafter each dog was given a pan containing 400 cc. of the mixture of milk and water. The signal was repeated four times to each dog while the dog was drinking. After that the dogs stayed quietly in their places for three hours, and during this period all disturbances were kept at a minimum. Three hours after drinking they were again catheterized and returned to their metabolism cages. The quantity and specific gravity of the urine obtained were determined. The course of these experiments was interrupted once or twice a week. On these days the dogs were taken into the experimental room but were kept fasting, and at the end of three hours they were catheterized also. The preceding feeding of these animals was at 5:00 p.m. on the day before, so that the urine obtained during these experiments represents that excreted when the dogs were fasting, between fifteen and eighteen hours after the last intake of fluid. In these dogs, kept on a normal diet and intake of fluid, the urine obtained when the animals were fasting varied from 20 to 50 cc., with a specific gravity of from 1.016 to 1.034.

After these experiments had been carried on for six weeks it was thought that the frequent drinking, under particular conditions, might have led to the establishment of a conditioned reflex so that diuresis would result without the administration of fluid. Accordingly, one or two days a week the empty milk pan was placed before the dog, and at the same time the musical signal was given. This expectation was not fulfilled during the first five months. The administration of 20 cc. of the mixture of milk and water, which the dog licked up from the pan, was likewise without effect. Although there was no effect on diuresis, other conditioned reflexes appeared, one dog in particular showing a marked salivary response to the musical signal.

The behavior of the dogs was of special interest on the days when mock-drinking was used to test the development of a conditioned reflex. At first the empty pans were licked out, and the dogs were restless and disturbed during the experimental period. After about three weeks a change in behavior was noticed in one dog, a quiet collie with a placid disposition. After licking out the empty pan she lay down quietly, as was her habit after the regular drinking test. Two of the other dogs later showed the same behavior; the fourth, however, apparently never accepted the experimental condition.

After the experiments had been in progress for five months they were

interrupted for a period of three weeks. Ordinarily one would expect such an interruption to destroy the effect of previous training. It has been observed, however, by Pavlov and Volberth that such a rest period usually reinforces a conditioned reflex much more than prolonged training. This apparently was true in the dog considered here. On the occasion of the first mock-drinking, three days after the experimental routine was started diuresis was marked. There was a urinary output of 180 cc. with a specific gravity of 1.007, although fluid had not been given. This diuretic response was readily obtained on this dog thereafter. The other possible causes of diuresis, such as changes in the temperature of the room, irregularities in intake of fluid, and so forth, could be excluded. It seems probable, therefore, that the diuresis was of reflex origin, conditioned by the experimental procedure.

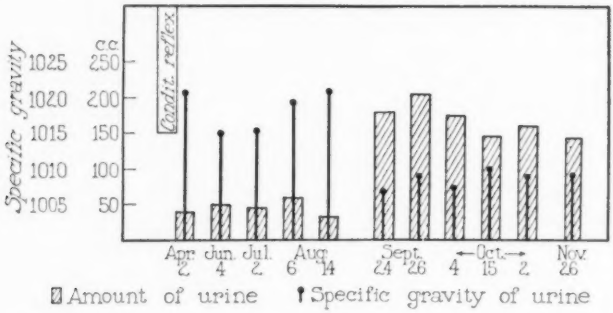

Fig. 1. Output of urine of a dog over a period of seven months, in which marked increase over the normal output was produced by a conditioned reflex.

The quantity of urine obtained, from 145 to 205 cc., was the same as that obtained in the water-drinking test in this dog. Further experiments are necessary to decide whether this is also an effect of the conditioned reflex or is an accidental result. Reflex diuresis appeared regularly in this dog alone, although a slight and variable diuretic response developed in a second dog after about eight months of training. Figure 1 shows the development of the reflex diuresis in the mock-drinking test; one may note the result of interruption in the course of experiments. It also shows the variation in the amount of urine obtained, which became somewhat less with the passage of time. This variation may be connected with possible fatigue of the reflex, for the dog became nervous and irritable during the last weeks of the experiment, a condition which had not been observed previously. This again is in harmony with the observations of Pavlov (1928), who noted the appearance of distinct neurotic disturbances in many of his animals in which conditioned reflexes had been studied over a prolonged period.

It seemed probable that the reflex diuresis was conditioned, not by the musical signal alone, but by the entire experimental procedure, and so was determined by a complex stimulus rather than by a single stimulus. After the appearance of psychogenic diuresis had been established, an effort was made to analyze the effect of single factors. It is difficult to analyze the different psychologic factors involved in an experiment such as this. It is recognized that the effect is determined by the experimental conditions as a whole, and not by the result of the summation of the individual elements. Furthermore, it is impossible to eliminate one factor without introducing other, and probably disturbing, factors in its place.

I sought, however, to divide the complex stimulus and to determine, so far as possible, the individual effect of: 1, the room in which the experiment was carried out, *R*; 2, the person conducting the experiment, *P*; 3, the musical signal, *S*, and 4, the use of the milk sample, *M*. Changes in the urinary output, after elimination of one or the other of these factors, were assumed to indicate the quantitative effect of the factor concerned. The results of these experiments are shown in figure 2. Group *a* gives the result in which the complete procedure and the entire complex stimulus was used. Group *b* shows the quantity of urine obtained when the sample of milk in the pan was not given. The quantity of urine (about 50 cc.) was distinctly less, and the specific gravity (from 1.010 to 1.012) was higher than with the complete procedure. Almost the same quantity of urine was obtained in group *c*, in which milk was given, but in which the musical signal was omitted; the specific gravity also was approximately the same as in group *b*. The results were much more variable in group *d*, in which both the milk sample and the musical signal were omitted. On one occasion 100 cc. of urine with a specific gravity of 1.015 were obtained, and on another occasion, only 45 cc. with a specific gravity of 1.023 were obtained. The latter is a normal resting value comparable to the control experiments. In group *e*, in which the experiment was carried on in the usual place, but in which the sample of milk was given by another person, diuresis did not occur. The experiments in groups *b* and *c* were repeated in a different room with essentially the same results. Diuresis was not obtained in control experiments (group *f*) in which the dog was alone in the room, and no other stimulus was given.

In the ordinary drinking test there is a simple relation between the intensity of the diuresis and the intensity of the stimulus, the quantity of urine excreted being proportional to the quantity of water administered. This relation does not hold in experiments such as these, which have been carried out on the fasting animal, and in which the excreted water must come from the reserve supplies of water in the organism. Under these conditions a doubling of the volume of urine necessarily implies a great

increase in the strength of the stimulus for diuresis. The fact that the diuretic response in group *a*, in which all the factors were present, was almost twice as great as the response in any of the other groups, in which individual factors were omitted, would, therefore, indicate that there was a much more than proportional increase in the stimulus.

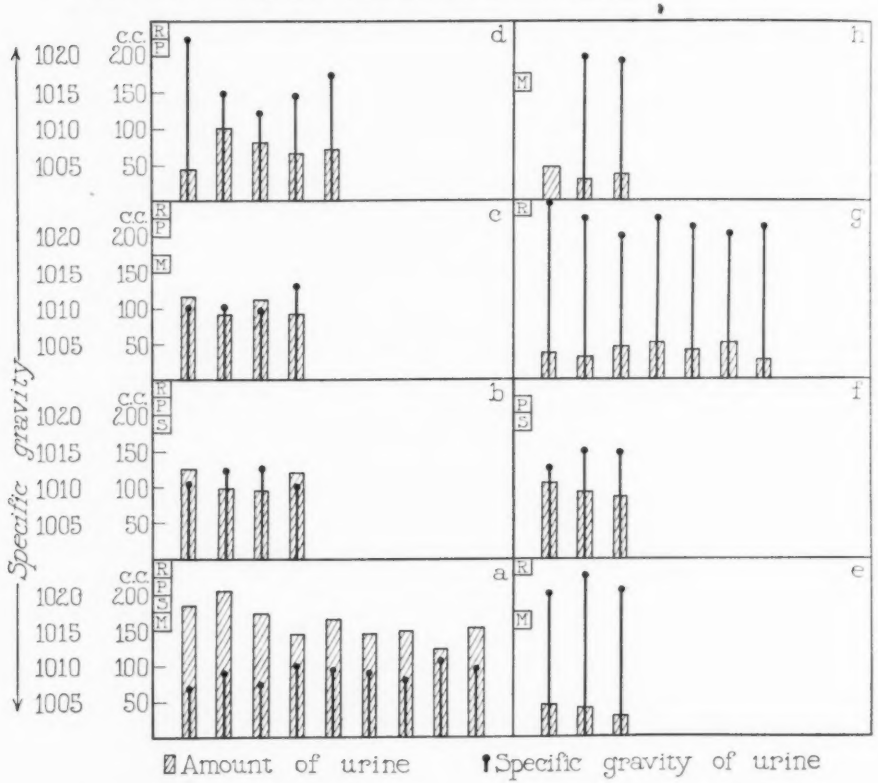

Fig. 2. The diuretic response to a conditioned reflex in which the component parts of the complex stimulus were applied separately: Room in which the experiment was carried out, *R*; *P*, person conducting experiment, *S*, musical signal, and *M*, milk.

The effect of the condition of the reserve supplies of water in the organism in determining the response to a diuretic stimulus is also shown by an experiment in which the fasting dog was kept in the room at a temperature of 92°F. Under these conditions the response, both to the ordinary drinking test and to reflex diuresis, was greatly reduced, as is shown in figure 3.

These experiments were not carried out under such rigid conditions as

suspension of the dogs in a darkened room, and so forth, which Pavlov (1927) insisted are necessary for the uniform production of conditioned reflexes. During these experiments, the dogs were maintained under approximately normal conditions. Furthermore, from the standpoint of temperament and disposition, the animals were not especially selected for these experiments. These factors will perhaps explain why reflex diuresis only appeared after several months of training and then in only one of four dogs. It is also possible that reflex diuresis is much more difficult to produce than other conditioned reflexes, such as the salivary reflex, for the latter appeared within six weeks in one of these dogs and finally appeared in all four animals.

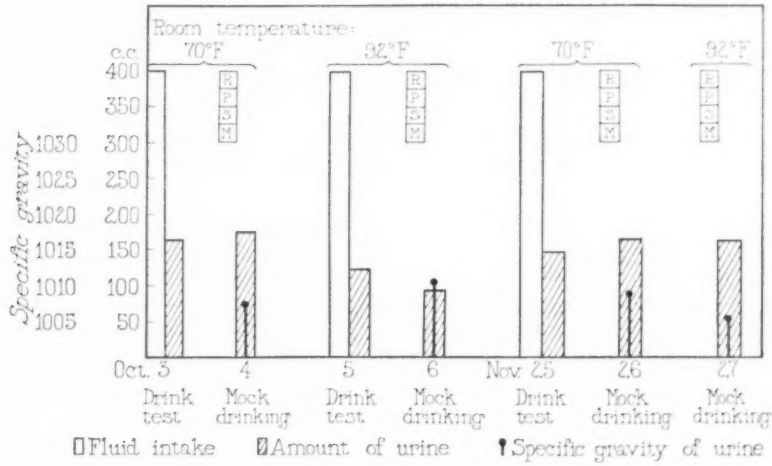

Fig. 3. The effect of room temperature on the output of urine, following administration of fluid by stomach tube and the effect of room temperature on the reflex diuresis.

CONCLUSION

In these experiments, reflex diuresis was obtained as a result of psychic stimuli. I believe that this demonstration is of twofold significance: as evidence for the importance of cerebral mechanisms in the regulation of diuresis, and in providing a method for the study of psychic mechanisms whereby it is possible to obtain a quantitative measure of the effects produced.

BIBLIOGRAPHY

BAILEY, P. AND F. BREMER. 1921. Arch. Int. Med., xxviii, 773.
BAUER, J. 1928. Ann. Int. Med., ii, 127.

VON BECHTEREW, W. 1905. Arch. f. Physiol., 297.
BOURQUIN, H. 1927. This Journal, lxxix, 362.
CAMUS, J. AND G. ROUSSY. 1913. Compt.-rend. Soc. de biol., lxxv, 483.
CURTIS, G. M. 1924. Arch. Int. Med., xxxiv, 801.
HOFF, F. AND P. WERMER. 1926. Arch. f. exper. Path. u. Pharm., cxix, 153.
JUNGMANN, P. AND E. MEYER. 1913. Arch. f. exper. Path. u. Pharm., lxxiii, 49.
MARX, H. 1926. Klin. Wochenschr., v, 92.
PAVLOV, I. P. 1927. Conditioned reflexes. London, Oxford University Press, 430 pp.
1928. Lectures on conditional reflexes. New York, International Publisher, 414 pp.
UCKO, H. 1923. Zeitschr. f. d. gesammt. exp. Med., xxxvi, 311.
VERNEY, E. B. 1929. Lancet, i, 539.
VOLBERTH, H. Personal communication to the author.

THE ACTIVATION OF DIFFERENT ELEMENTS OF THE GASTRIC SECRETION BY VARIATION OF VAGAL STIMULATION

ARTHUR M. VINEBERG

From the Department of Physiology, McGill University, Montreal, Canada

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The fact that the vagus nerves are the true secretory nerves of the peptic glands has been well established. However, the relation of these nerves to the different cytological elements comprising the gastric mucous membrane is not entirely clear. In the light of our present knowledge it would seem that the structure of the gastric mucous membrane exhibits at least four different histological elements: 1, the surface epithelial cells; 2, the chief neck cells or mucoid cells (Bensley, 1898; Lim, 1922; Aschoff, 1923; Zimmermann, 1925); 3, the chief body cells or peptic cells; 4, the parietal cells or oxyntic cells.

The influence of the vagus nerve on the peptic and parietal cells has already been indicated by Pavlov and other investigators of his school. However, the relation of the vagus fibres to the other cytological elements of the gastric mucous membrane is still somewhat obscure, although there are indications that the vagus also exerts a certain influence on these cells. Opinions differ on this point (cf. Oushakov, 1896; Bickel, 1910; Savitsch, 1922; Anrep, Pavlov and Savitsch, 1922; Stahnke, 1924). However, according to Oushakov, Savitsch *et al.* different types of gastric juice may be obtained by vagal stimulations of different character. For example, the juice from sham feeding is high both in peptic power and acid content, whereas the juice from an acute experiment is extremely rich in pepsin and has a low total acidity.

In the present investigation an attempt has been made to correlate previously known facts by applying induction currents of different strengths to the vagi in dogs, with the object of obtaining gastric secretions of different composition.

METHODS. Dogs of medium size were used. Care was taken to ensure that each animal received adequate nourishment beforehand, but yet had an empty stomach on the day of the experiment. The operative procedures described were executed as quickly as possible.

Following a short ether anesthesia, a mixture of chloralose (0.05 gram per kilo) and urethane (0.5 gram per kilo) was slowly injected intravenously. Tracheotomy was then performed and the esophagus tied in the neck. The vagi were isolated and

severed as high in their course as possible. Through a mid-line upper abdominal incision, a metal fistula was inserted into the anterior surface of the stomach. The pylorus was tied at the pyloro-duodenal junction. In some of the experiments the esophagus was tied just below the diaphragm without injury to the vagal plexus in this region, while in others a cannula was inserted into the lower end of the esophagus. The stomach was returned to its natural position of rest, and the abdominal wall closed about the metal fistula.

At the conclusion of the operation the animal was placed in a prone position on a stand specially designed with a central window through which the gastric fistula projected. The animal was kept warm throughout the experiment.

The vagi nerves were placed on platinum electrodes and stimulated alternately and rhythmically (15 interruptions per minute by Meltzer's metronome) for a period of ten minutes each. The induction coil employed was of the A. H. Baird (Edinburgh) type, and was calibrated to give the following currents for the corresponding centimeter readings on the scale: 11 cm., 0.20 milliampere; 10 cm., 0.50 m.a.; 9 cm., 1 m.a.; 8 cm., 1.80 m.a.; 7 cm., 2.90 m.a., etc. At the beginning the electrodes were always applied at the central end of the severed vagi and during the course of the experiment were gradually moved along toward the peripheral end of the nerves.

When the methods of anesthesia and operative procedure outlined above were strictly adhered to, vagi stimulation in almost every case gave positive results. In our series of experiments, out of a total of twenty-five experiments attempted twenty-four were entirely satisfactory.

The digestive power was determined by means of Metts' method as modified by Hawk and Bergeim (1927). Free and total hydrochloric acid were determined by the titration method, using Töpfer's reagent and phenolphthalein as indicators, while the chlorine content was estimated by the recent method of Wilson and Bald (1928). The volume of visible mucus was ascertained by centrifuging each sample of juice at 1700 revolutions per minute for a period of ten minutes.

EXPERIMENTAL RESULTS. The chief point established in this investigation is that the characteristics of the gastric juice obtained after rhythmic stimulaton of the vagi in the neck differ greatly according to whether a weak or strong induction current is used. The experiment shown in table 1 may be taken as illustrative of this fact. The following are some of the conclusions which may be drawn from the experiment and which are representative of the results obtained in this series of analogous experiments. First, under the conditions of an acute experiment a weak rhythmic stimulation of the vagi activates a secretion of alkaline mucus. The digestive power is low, and also the chlorine content. (By weak stimulation is meant a current of 0.50 milliampere or less, and one which is barely perceptible to the human tongue.) Second, with a strong current (i.e., a current of 1.0 milliampere or more, and one which is decidedly unpleasant when applied to the human tongue) the digestive power increased greatly. Now, since it is known that the mucoid secretion from the surface epithelial cells does not contain pepsin, it seems reasonable to assume in the experiment in question that some neutral or alkaline secretion probably of mucoid nature, secreted by the peptic glands, was added to the mucoid secretion (sample 2, table 1). Third, when a strong current (9 cm.) is applied to the

TABLE 1

March 20.—Dog, weight 13 kilos, under chloralose and urethane anesthesia. No artificial respiration. Esophagus tied in the neck; pylorus tied at the junction with the duodenum.

Stomach practically empty; gastric contents only 1.7 cc. (sample 1). Reaction slightly acid. Stimulation of vagi (coil 9 cm.) at 2:50 p.m. First drop of acid juice at 3:10 p.m. Coil 7 cm. at 4:35 p.m.

SAMPLE	TIME		GASTRIC SECRETION						STIMULATION OF VAGI	
			Volume	Free HCl	Total HCl	Mucus volume	Chlorine	Peptic activity	Nerve	Coil
		minutes	*cc.*	*gram per cent*	*gram per cent*	*cc.*	*mgm. per cent*			*cm.*
1			1.7	0.02	0.11	0.00		134.5		
2	10:30–11:30 a.m.	60	3.0	0.00	0.06	0.00	452.3	369.9	Right and Left	13
3	11:30–12:00 noon	30	1.0			Trace			Right and Left	13
4	12:00–12:30 p.m.	30	4.0	0.00	0.01	1.5	429.6	16.0	Right and Left	13
5	12:30– 1:00 p.m.	30	5.0	Alkaline		2.0	469.0	16.0	Right and Left	13
6	1:00– 1:30 p.m.	30	3.5	Alkaline		2.5	331.8	16.0	Right and Left	11
7	1:30– 2:00 p.m.	30	5.0	Alkaline		4.9			Right and Left	11
8	2:00– 2:30 p.m.	30	2.5	Alkaline		2.2	331.8	89.4	Right and Left	10
9	2:30– 3:00 p.m.	30	3.0	Alkaline		3.0	334.6	36.0	Right and Left	9
10	3:00– 3:30 p.m.	30	5.5	Alkaline		5.0	336.0	64.0	Right and Left	9
11	3:30– 3:45 p.m.	15	3.0	0.00	0.27	3.0	453.7	1632.0	Right and Left	9
12	3:45– 4:00 p.m.	15	4.9	0.11	0.43	3.0	472.0	1830.4	Left and Right	9
13	4:00– 4:10 p.m.	10	6.5			1.0			Left	9
14	4:10– 4:20 p.m.	10	Lost	—	—	—	—	—	Right	9
15	4:20– 4:30 p.m.	10	2.4	0.29	0.47	0.3	523.2	1324.9	Left	9–7
16	4:30– 4:40 p.m.	10	2.5			0.4			Right	7
17	4:40– 4:50 p.m.	10	3.0	0.27	0.52	0.5	506.1	1324.9	Left	7
18	4:50– 5:00 p.m.	10	1.8			0.3			Right	7

vagi, gastric juice of normal composition is produced. It is high in acidity and has a maximal concentration of chlorine and an extraordinarily high peptic power.

The effect of weak or strong rhythmic stimulation of the vagi in the neck on the composition of the gastric juice is a constant phenomenon. In dogs anesthetized with the chloralose and urethane mixture the application of an induction current of 9 cm. (1 milliampere) to the vagus nerves invariably produced a flow of gastric juice in from *seven to forty-two minutes.* The average latent period of gastric secretion in a series of nineteen experiments with a current of 9 cm. was twenty-two minutes.

Analysis of the results obtained. The vagal juice obtained by previous investigators in the acute experiment was usually low in acid content, rich

TABLE 2

Dog, weight 7.8 kilos, under dial injected intraperitoneally. Artificial respiration used throughout the experiment. Alternating rhythmic stimulation of the right and left vagi in the neck by a gradually increasing current, coil at 16 to 11 cm.

SAMPLE	TIME	GASTRIC SECRETION				
		Volume	Free HCl	Total HCl	Mucus	Peptic activity
	minutes	*cc.*	*gram per cent*	*gram per cent*		
1	30	1.6	0.05	0.07		19.3
		Stimulation of vagi, current 16.0—11 cm.				
2	10	5.2	0.01	0.02		20.8
3	120	0.5	0.00	0.00	Jelly-like	4.0
4	60	1.7	0.00	0.00	Jelly-like	10.0
5	60	2.8	0.00	0.05	Stringy	353.4
6	30	2.5	0.00	0.02	Stringy	163.8
7	30	3.1	0.00	0.02	Stringy	353.4
8	30	1.8	0.00	0.02	Stringy	338.5
9	30	2.0	0.00	0.02	Stringy	312.8

in mucus and high in peptic power. In studying their technique we find that:

1. A. As weak a current as possible was used at the commencement of the experiment to avoid damaging the nerves and the heart.

B. The current was increased slowly and with extreme caution.

2. In most preparations the spinal cord of the animal was cut below the medulla. This procedure of necessity required the use of artificial respiration.

In the light of our own work it would seem very probable that the great amount of mucus obtained by our predecessors was due to the weakness of the stimulation used.

Table 2 represents an experiment in which weak stimulation was applied

(coil 16 to 11 cm.) and artificial respiration was used. The secretion, which at the outset was acid over a period of three hours, became definitely alkaline and composed of a jelly-like mucoid substance. The digestive power was extremely low (samples 3 and 4). When the current was increased the secretion increased in volume and became faintly acid. At no time was there free acid. The peptic power increased at once from 10 to 353.4 Metts' units. The secretion still retained its mucous character, but the formerly thick gelatinous mucus became stringy and clotted. As there was an increase in the volume of the secretion the rise in the peptic power must be considered as due to a true secretion of pepsin.

TABLE 3

September 19.—Dog, weight 12.1 kilos, under chloralose and urethane anesthesia. No artificial respiration. Esophagus tied in the neck and below the diaphragm; body of stomach isolated from pylorus as described above.

SAMPLE	TIME	GASTRIC SECRETION					STIMULATION OF VAGI	
		Volume	Free HCl	Total HCl	Mucus volume	Peptic power	Nerve	Coil
	minutes	*cc.*	*gram per cent*	*gram per cent*	*cc.*	*Metts' units*		*cm.*
1	90	2.0	0.02	0.17	Trace	64.0		
2	60	2.5	0.00	0.14	2.4	64.0	Right and Left	14
3	60	1.6	0.00	0.05	1.6	36.0	Right and Left	14
4	60	1.5	0.00	0.03	1.5	16.0	Right and Left	14
5	60	1.8	0.00	0.001	1.8	36.0	Right and Left	13.5
6	60	1.5	0.00	0.001	1.5	36.0	Right and Left	13.5
7	60	2.4	0.00	0.001	2.4	100.0	Right and Left	9.0
8	30	2.7	0.05	0.18	0.4	324.0	Right and Left	9.0
9	30	1.6	0.09	0.23	1.0	354.0	Right and Left	8.5
10	30	4.8	0.14	0.28	4.0	576.0	Right and Left	8.5
11	30	5.0	0.18	0.34	4.0	784.0	Right and Left	8.5
12	20	8.5	0.25	0.39	5.0	806.5	Right and Left	8.5
13	20	8.4	0.24	0.40	4.0	900.0	Right and Left	8.5

This mucus obtained in our experiments by weak stimulation has four possible sources: 1. The esophagus. 2. The pyloric part of the stomach. 3. The surface epithelium of the fundal part of the stomach. 4. The mucoid cells of the peptic glands.

The esophagus as a source of mucus was excluded in the following manner. A cannula was inserted into the lower end of the esophagus below the diaphragm without injuring the branches of the vagi nerves (see under "methods"). A second cannula was tied into the distal end of the esophagus in the region of the neck. Under such conditions weak stimulation of the vagi caused a flow of mucus from the stomach and none from the esophagus. Strong stimulation, on the other hand, initiated a

copious flow of acid juice from the stomach. In addition a considerable amount of thick gelatinous transparent alkaline mucus was obtained separately from the esophagus. Thus mucus obtained under the influence of weak stimulation comes from the stomach and not from the esophagus.

The chief source of the mucus secreted during stimulation of the vagi was found to be the fundal part of the stomach. This was proved in the following manner. The esophagus was tied below the diaphragm and the pyloric antrum isolated from the remaining part of the stomach. In order to accomplish the latter operation a small longitudinal incision was made on the anterior surface of the pyloric part of the stomach at a distance of 4 to 5 cm. from the pyloric sphincter. The muscular coats alone were sectioned. By careful dissection the submucous mucosa was carefully separated from the muscularis throughout the entire circumference of the pyloric antrum. The isolated submucosa and mucosa were then tied securely. By this method the pyloric part was isolated from the remainder of the stomach with a minimal amount of traumatization and without entering into the lumen of the organ.

Table 3 represents an experiment in which the above procedure was followed. In spite of the absence of the pyloric part of the stomach, i.e., the part secreting alkaline mucoid fluid containing pepsin, there was a flow of mucus after stimulation with a weak current. From a study of this experiment it will be observed that over a period of six hours there was a steady flow of mucus. The reaction of this secretion was faintly alkaline or neutral to litmus paper, but it contained a faint trace of acid when titrated. It is interesting to note that the original contents of the stomach collected during a period of 90 minutes contained only a trace of mucus with fairly high acidity. This suggests that the traces of acid in samples 2, 3, 4, 5, 6, and 7 represent simply a washing out of the original acid content by freshly secreted mucus. Further, when a strong stimulus was applied, there was a flow of free acid within thirteen minutes. This occurred after six hours of weak stimulation. The secretion thus obtained gradually increased in volume, acidity and peptic power to values comparable with those obtained in other experiments in which strong stimulation was applied.

From the afore-mentioned experiment it is apparent that mucus can be obtained by weak stimulation from the fundus or body of the stomach. The most difficult point to establish is the exact source of the mucus secreted during stimulation of the vagi, in relation to the histological structure of the stomach. Under the conditions of stimulation a certain proportion of the total mucus secreted must be contributed by the surface epithelium, particularly if the stimulation of the vagi influences the motility of the gastric mucous membrane. In order to ascertain whether this was true or not, the following experiment was performed.

The animal was prepared in the usual manner. A gastric fistula was inserted into the posterior surface of the stomach in lieu of the anterior surface and brought out through a stab wound in the left flank. A glass window was sewed into the anterior surface of the stomach in such a manner as to permit observation of part of the gastric mucous membrane during the experiment. Subsequent to stimulation of the vagi not only a flow of gastric juice but also definite movements of the gastric mucous membrane were observed.

Thus the mucus of the surface epithelium may be washed out by the flow of gastric juice and also pressed out by the movements of the mucous membrane. Further, it is possible that the vagi have a direct action on the secretory elements of the surface epithelium. There are, however, certain facts which indicate that the mucoid secretion may arise from another source. For example, 1, under vagal stimulation the mucus secreted was obtained in larger volumes and had a more liquid character than the mucus of surface epithelial origin. 2. When this mucus was properly diluted with a N/20 solution of HCl it possessed a high peptic power. The presence of a high peptic content usually coincided with the increased flow of mucus and the appearance of the first trace of acid secretion. Such was the picture when weak stimulation was applied to the vagus nerves. A strong current activated a secretion of gastric juice which was high in acid and enzyme content but poor in mucus. 3. Webster (1930), working in our laboratory, found a mucin-like substance present in the gastric juice in a soluble state. Its concentration ran parallel to that of the peptic power of the juice.

Hence it might not be unreasonable to suppose that the mucus which possessed digestive enzymes was secreted by special mucoid cells located in the neck of the peptic gland. However, in order to establish the truth of the hypothesis just enunciated, a combined histo-physiological investigation of the mucous membrane of the stomach during different phases of its activity would be required.

Discussion. In this investigation it has been demonstrated that it is possible to obtain two types of gastric secretion following the application of currents of different strength to the vagus nerves in the neck. On the one hand, a mucoid secretion was produced in response to *weak stimulation*, and on the other hand gastric juice of normal composition was produced in response to *strong stimulation*. The possible explanations of the mechanism involved are manifold. The most feasible seems to be that there are different nerve fibres in the vagus supplying different gastric secretory elements, and that each fibre or group of fibres is affected by currents of different intensity. In considering the effect of strong stimulation of the vagi it is interesting to note that the gastric secretion thus activated possessed a higher peptic content than the gastric juice obtained from dogs

with esophagotomy and gastric fistula or with a Pavlov pouch. Therefore it would seem that the vagus nerve, in addition to its power of activating the parietal cells, bears a definite relation to the pepsin-producing cells of the gastric gland. The vagus nerve might thus be regarded as the true "trophic" nerve of the gastric mucosa.

To interpret the part played by the gastric mucus in the stomach is by no means easy, nor is it less difficult to comprehend the significance of its activation by vagal stimulation. There are two main theories concerning the function of gastric mucus in relation to the stomach. The first is that the gastric mucus is a protective agent for the gastric mucosa (see Babkin, 1928). The second is the theory subscribed to by Brestkin and Bykoff (1924), who maintain that mucus mixed with gastric juice goes partly into solution and thereby raises the digestive power of the juice. Again, it is possible that mucus may play some part in certain pathological conditions. For example, there might presumably be some correlation between the formation of peptic ulcers and the function or lack of function of the mucoid cells of the gastric mucosa. Clinically, Kaufman (1908), after analysing many cases, came to the conclusion that free microscopical mucus was decidedly lacking in cases of peptic ulcer. He explained the relief of pain following gastric lavage with a solution of silver nitrate to the excessive amount of mucus thrown out in response to a local irritant. Further, Volkovitch (1898) in Pavlov's laboratory studied a case of spontaneous gastric ulcer which formed in an isolated Pavlov pouch in a dog. He found that after feeding there was no change in the first or nervous secretory phase, but that there was a great prolongation of the second or chemical phase. In addition the acidity of the juice was above normal, while the peptic content was somewhat lower. Thus it would seem that in the presence of peptic ulcers (in the dog) there is a hypersecretion of gastric juice, but the juice is poor in organic substances and in mucus; that is, it differs in composition from the normal and yet remains highly active. The elements of gastric secretion are thus pathologically separated, and produced independently in much the same manner as we have succeeded in producing different types of secretion by electrical stimulation.

SUMMARY

1. A satisfactory method has been evolved for obtaining a gastric secretion in a dog under anesthesia through the application of rhythmical stimuli to the vagi in the neck.

2. A weak induction current applied to the vagi in the neck stimulates a flow of mucus having a high digestive power.

3. The chief source of the mucus appears to be the mucous membrane of the body of the stomach, since it was obtained following the exclusion of the mucoid secretions from the esophagus and from the pylorus.

4. A strong induction current provokes a flow of gastric juice with a very high digestive power, high acidity, normal content of chlorine and a small amount of mucus.

5. It is suggested that the vagus nerve contains different fibres innervating different cytological elements of the gastric mucosa and that these may be activated by induction currents of different strengths.

This investigation was carried out under the direction of Dr. B. P. Babkin, to whom the writer is indebted for much helpful advice and criticism.

BIBLIOGRAPHY

ASCHOFF, L. 1923. Pflüger's Arch., cci, 67.
ANREP, G. V., V. I. PAVLOV AND W. W. SAVITSCH. 1922. Berichte der Lesgaftschen Institut, Petrograd, v, 53.
BABKIN, B. P. 1928. Die äussere Sekretion der Verdauungsdrüsen. Berlin. 2nd ed., p. 364.
BENSLEY, R. R. 1898. Quart. Journ. Micr. Sci., xli, 361.
BICKEL, A. 1910. Oppenheim's Handbuch d. Biochem., iii, part i, 55.
BRESTKIN, M. P. AND K. U. BYKOV. 1924. Journ. Russe de Physiol., vii, 381.
HAWK, P. B. AND O. BERGEIM, 1927. Practical physiological chemistry. 9th ed., p. 234.
LIM, R. K. S. 1922. Quart. Journ. Micr. Sci., lxvi, 187.
KAUFMAN, J. 1908. Amer. Journ. Med. Sci., cxxxv, 207.
OUSHAKOV, V. G. 1896. Dissertation, St. Petersburg.
SAVITSCH, W. W. 1922. Berichte der Lesgaftschen Inst., Petrograd, v, 45.
STAHNKE, E. 1924. Arch. f. klin. Chirurgie, cxxxii, 1.
VOLKOVITCH, A. N. 1898. Dissertation, St. Petersburg.
WEBSTER, D. R. 1930. Proc. Roy. Soc. Canada, Section V, 199.
WILSON, D. W. AND E. G. BALD. 1928. Journ. Biol. Chem., lxxix, 221.
ZIMMERMANN, K. W. 1925. Ergebn. d. Physiol., xxiv, 281.

STUDIES IN THE PHYSIOLOGY OF VITAMINS

XV. Some Observations of the Effect of Administration of the Antineuritic and Heat Stable Factors on the Anorexia Characteristic of Lack of the Vitamin B Complex

GEORGE R. COWGILL, H. A. ROSENBERG and J. ROGOFF[1]

From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

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One of the phenomena characterizing continued subsistence on a diet deficient in the vitamin B complex is loss of the urge to eat the incomplete ration. Administration of sufficient amounts of products rich in the missing vitamin results in a prompt correction of this anorexia (Karr, 1920; Cowgill, 1921; 1923a; Cowgill, Deuel and Smith, 1925). In previous papers of this series will be found reports of quantitative studies of this phenomenon as well as observations of gastro-enteric functions and blood changes in vitamin B deficiency as exhibited in dogs. As a result of these numerous and varied observations we have been led to believe that the appearance of this characteristic subtle loss of appetite for the experimental ration is in most cases the first definite sign that a state of vitamin B deficiency exists in the experimental animal. Using this anorexia as such a criterion, measurements of the vitamin B requirements of numerous different-sized individuals of different species—mouse, rat, pigeon and dog—have been made in this laboratory in the hope that from the data thus obtained it might be possible to find some common relationships of the vitamin requirement to weight or other functions of size from which it might be reasonable to generalize to the human species. In this way it was felt that one might eventually secure some elucidation of the factors that operate to determine the vitamin B requirement in a given human being.

Since the beginning of the studies reported in this series of papers numerous investigators have brought forward evidence showing that what has hitherto been called vitamin B really consists of two or more physiologically active substances (S. Smith, 1928). At the present time two components of the vitamin B complex are generally recognized.

[1] This paper is a report of some of the data contained in the thesis submitted jointly by Harold Arthur Rosenberg and Jacob Rogoff to the faculty of the Yale University School of Medicine in April, 1930, in partial fulfillment of the requirements for the degree of Doctor of Medicine.

These have been variously denominated (*a*) the antineuritic, heat labile substance, vitamin B or B_1 or F, and (*b*) the antipellagric, heat stable factor, which has also been called vitamin B_2 and vitamin G. More recently reports have been published suggesting the existence of still another active substance in the vitamin B complex (Williams and Waterman, 1928; Hunt, 1928; Reader, 1930; Eddy, Gurin and Kerestzkey, 1930). In view of these developments, and as an aid to the proper interpretation of our quantitative data, it becomes important to determine, if possible, the rôle of each of these well-recognized B factors in correcting the anorexia characteristic of so-called vitamin B deficiency.

Indirect evidence suggests that a lack of the antineuritic factor is primarily responsible for this anorexia. Bing and Mendel (1929) observed that mice receiving antineuritic vitamin B but no pellagra-preventive substance beyond that possibly present as impurity in the components of the experimental ration, showed no loss of appetite and maintained a more or less constant body weight until pronounced cutaneous symptoms developed. This was in marked contrast to the behavior of mice given autoclaved yeast but no antineuritic vitamin; these animals soon began to lose weight and declined rapidly. The work of M. I. Smith (1930) on rats, and that of Seidell (1922) and Klotz (1926) on pigeons, likewise suggests that the anorexia feature of the syndrome of vitamin "B" deficiency is to be attributed to a lack of the antineuritic factor.

For the performance of experiments designed to answer the question as to the rôles played by the respective components of the B complex in correcting this characteristic anorexia it is necessary to obtain preparations of the antineuritic and antipellagric vitamins, each as free as possible from the other. Through the courtesy of Drs. H. B. Vickery and L. B. Mendel, we were furnished with sufficient amounts of a fuller's earth adsorbate of the antineuritic factor prepared for them by Dr. C. N. Frey.[2] This had been tested on rats and found to require supplementing with autoclaved yeast in order to allow normal growth. Doctor Frey also reported that this material was effective in preventing the development of neuritis in young rats. Autoclaved yeast has been shown by many investigators to be a good source of antipellagric vitamin relatively free from the heat labile antineuritic factor (Smith and Hendrick, 1926; Chick and Roscoe, 1927; Sherman and Axtmayer, 1927). This paper is a report of the effect of the administration of the fuller's earth adsorbate just referred to and of autoclaved yeast on the anorexia occurring in dogs subsisting on a diet deficient in the vitamin B complex.

PLAN OF EXPERIMENTS. Dogs were allowed to subsist solely on the *casein III* (Cowgill, 1923b) diet that has been used so often in the vitamin studies conducted in this laboratory. This artificial food mixture is

[2] Research Laboratories of the Fleischmann Company, New York.

adequate for canine nutrition when it is supplemented by a source of the vitamin B complex. When this basal diet alone was fed and the characteristic anorexia had appeared after about three weeks, autoclaved yeast, fuller's earth adsorbate and a control substance devoid of either vitamin, namely, fuller's earth alone, were administered respectively, and their efficacy in restoring the urge to eat was observed.

DETAILS OF THE EXPERIMENTS. In the first two trials yeast that had been autoclaved for three hours at a temperature of 120°C. and a pressure of 15 pounds was used. Because these first tests gave a suggestion of a positive effect on the anorexia, and the indirect evidence bearing on the question pointed to the antineuritic vitamin as the substance required in order to correct this loss of appetite, it was thought possible that this yeast still contained an appreciable amount of the antineuritic substance. A second autoclaving of this sample under the same conditions that prevailed in the first treatment was therefore performed. Tests of this twice-autoclaved yeast on young rats indicated that it contained the antipellagric component of the B complex in amounts sufficient to allow growth.[3]

In the initial series of tests on four dogs the fuller's earth adsorbate was used first. An attempt to administer this material by stomach tube in the form of a water suspension met with so much difficulty due to clogging of the tube that this method was abandoned. In subsequent experiments the adsorbate was given in gelatin capsules. To only two dogs in this series of trials was autoclaved yeast administered. In these tests, as has already been stated, there was a suggestion of a positive effect in the correction of anorexia and it was deemed wise to autoclave the yeast a second time. In the second series of experiments four animals were again used. When anorexia developed in these dogs, autoclaved yeast was tested first, then fuller's earth, and finally the fuller's earth adsorbate.

RESULTS. *In every case—eight in all—following the administration of fuller's earth adsorbate, the anorexia was corrected.* In the two tests made with the first sample of autoclaved yeast there was a suggestion of a positive response. In all of the four subsequent tests made with the same yeast after re-autoclaving, however, the appetite was not restored. All four control tests with fuller's earth alone were likewise negative.

DISCUSSION. These results clearly indicate that the anorexia exhibited by dogs subsisting on our *casein III* diet is to be attributed to a lack of the antineuritic component of the B complex. This loss of appetite for the experimental ration is readily corrected by supplying antineuritic vitamin prepared from yeast; it is not influenced by supplying the heat-stable pellagra-preventive substance; neither is it corrected by the presence in the alimentary tract of an indifferent substance such as fuller's earth.

[3] Personal communication from Dr. Arthur H. Smith.

If a third unknown factor plays an indispensable rôle, then in these experiments it must have been present either in the adsorbate along with the antineuritic substance or as an unrecognized impurity in the basal ration.

Commercial casein was used as a source of protein in the basal diet here employed. Evans and Burr (1928) have shown that this material contains as an impurity appreciable amounts of the heat-stable antipellagric vitamin which is present in milk (Hunt and Krauss, 1928). The fact, that lesions suggestive of pellagra (Cowgill, Stucky and Rose, 1929) have occasionally been observed in dogs subsisting on the *casein III* diet, may be cited in support of the view that the amount of antipellagric vitamin present in the casein used here is variable. The conditions of the present experiments were not such as to allow one to say that a lack of the antipellagric factor plays no rôle in the correction of this characteristic anorexia. The dogs may have been receiving enough of this vitamin as an impurity present in the casein. Further experiments with animals fed a more highly purified ration are now in progress. The investigations of experimental pellagra thus far reported suggest that the body's capacity for storage of the antipellagric factor is greater than that for the antineuritic vitamin. Longer periods of subsistence on the deficient diet seem to be necessary in order to produce the *symptoms of pellagra*—not merely a suppression of growth—than is the case with experimental beriberi. These considerations reinforce the conclusion indicated by the results of the present experiments, namely, that the anorexia exhibited after three weeks or more by dogs subsisting on our *casein III* ration is a phenomenon attributable to lack of the antineuritic vitamin B.

It is apparent that the results of the quantitative studies of vitamin B reported from this laboratory in former years (Cowgill, Deuel and Smith, 1925; Beard, 1926; Cowgill and Klotz, 1927), in which this characteristic anorexia was used as the sign that a state of vitamin B deficiency exists, actually apply primarily to the antineuritic component of the vitamin B complex.

CONCLUSION

The anorexia that develops in dogs after about three weeks' subsistence on the *casein III* diet employed in the researches of this laboratory, is an expression of a lack of the antineuritic component of the vitamin B complex.

BIBLIOGRAPHY

Beard, H. H. 1926 This Journal, lxxv, 668.
Bing, F. C. and L. B. Mendel. 1929. Journ. Nutrition, ii, 49.
Chick, H. and M. H. Roscoe. 1927. Biochem. Journ., xxi, 698.
Cowgill, G. R. 1921. This Journal, lvii, 420.
1923a. Journ. Biol. Chem., lvi, 725.
1923b. This Journal, lxvi, 164.

COWGILL, G. R., H. J. DEUEL, JR. AND A. H. SMITH. 1925. This Journal, lxxiii, 106.
COWGILL, G. R. AND B. H. KLOTZ. 1927. This Journal, lxxxi, 470.
COWGILL, G. R., C. J. STUCKY AND W. B. ROSE. 1929. Arch. Path., vii, 197.
EDDY, W. H., S. GURIN AND J. KERESTZKEY. 1930. Journ. Biol. Chem., lxxxviii, 729.
EVANS, H. M. AND G. O. BURR. 1928. Journ. Biol. Chem., lxxvii, 231.
HUNT, C. H. 1928. Journ. Biol. Chem., lxxix, 723.
HUNT, C. H. AND W. E. KRAUSS. 1928. Journ. Biol. Chem., lxxix, 733.
KARR, W. G. 1920. Journ. Biol. Chem., xliv, 255.
KLOTZ, B. H. 1926. Determination of the vitamin B minimum in the pigeon. Thesis, Yale School of Medicine.
READER, V. R. 1930. Biochem. Journ., xxiv, 1172.
SEIDELL, A. 1922. U. S. Pub. Health Repts., U. S. P. H. S., xxxvii, 1519.
SHERMAN, H. C. AND J. H. AXTMAYER. 1927. Journ. Biol. Chem., lxxv, 207.
SMITH, M. I. 1930. U. S. Pub. Health Repts., U. S. P. H. S., xlv, 116.
SMITH, M. I. AND E. G. HENDRICK. 1926. U. S. Pub. Health Repts., U. S. P. H. S., xli, 201.
SMITH, S. L. 1928. Journ. Home Econ., xx, 241.
WILLIAMS, R. R. AND R. E. WATERMAN. 1928. Journ. Biol. Chem., lxxviii, 311.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE ORGANS

XXV. The Mystery of Emotional Acceleration of the Denervated Heart after Exclusion of Known Humoral Accelerators

H. F. NEWTON,[1] R. L. ZWEMER[2] AND W. B. CANNON

From the Laboratories of Physiology in the Harvard Medical School

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For more than a decade the denervated heart has been used in this laboratory to demonstrate alterations in the circulating blood, which offers the only medium uniting the organ with the rest of the body. At first the preparation was employed in anesthetized animals, but later, by use of careful surgical technique, a lasting preparation was devised (Cannon, Lewis and Britton, 1926) that permitted records to be made of the cardiac responses to changes in the blood in animals which survived for indefinite periods the denervation and which were subjected to various experimental tests. Thus when animals exercised moderately or manifested emotional excitement (Cannon and Britton, 1927), or reacted to external cold (Cannon, Querido, Britton and Bright, 1927), a special discharge of adrenin was demonstrated; for if the adrenal glands were excluded (by removal of one and denervation of the other), the same procedures did not induce the quick cardiac acceleration seen when the glands could function. Although after exclusion of medulliadrenal secretion the *immediate* acceleration disappears, as the published records prove (cf. Cannon and Britton, 1927), there is a belated acceleration which begins shortly after a struggle (e.g., for one minute) and which reaches a maximum usually three or four minutes after the struggle has ceased. This slow rise of the the rate of the denervated heart has been known at least since 1926. It has not been hitherto reported because it could not be explained. Much time and attention have been given to the search for an explanation. While searching we have commonly referred to the mysterious cardio-accelerator as the "x-factor." In this paper our negative experiments and the indicative evidence concerning the x-factor are presented. In the paper which follows is offered the positive testimony as to its source and nature.

[1] Workman Fellow in Physiology, Harvard Medical School.

[2] National Research Fellow in the Biological Sciences.

THE CHARACTER OF THE SLOW ACCELERATION. Examples of the acceleration of the heart after it has been isolated from the nervous system (by section of the right vagus below the recurrent branch, severance of all the cardiac connections of the left vagus, removal of the upper thoracic sympathetic strands), and after exclusion of hepatic and medulliadrenal

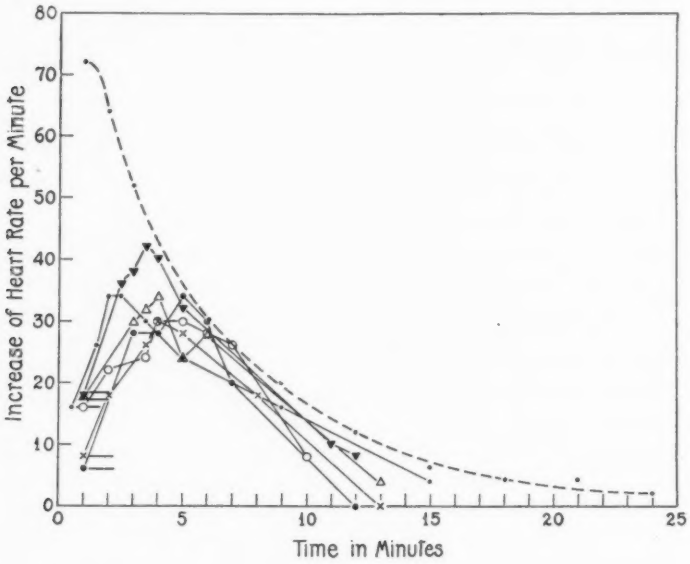

Fig. 1. Graphs (in solid lines) showing typical examples of the slow increase and decrease of the heart rate in cats after complete recovery from denervation of the heart and denervation of the adrenals and liver. The base line represents the original heart rate while the animals were comfortable on a cushion; the ordinate at 0 indicates when the animals had been transferred from the cushion and fastened back-downward on the holder; the short horizontal line at the right of the first record of heart rate shows the period of struggle.

In the upper graph (in a dash-line) is shown the immediate reaction of the denervated heart to one minute of excitement and struggle when the innervation of the adrenal glands is intact. The ordinate at 0 marks the transfer of the animal from the cage, in which it was excited, to a comfortable cushion (see Cannon and Britton, 1927).

influences (by upper abdominal sympathectomy, cutting of the liver nerves and the left splanchnics, and removal of the right adrenal) are seen in figure 1. These examples are taken from many similar records made on more than a score of cats. We wish to emphasize the facts that though the operations performed on some of the animals were ex-

tensive, they were done in stages, after complete recovery of vigor in each instance, and that always the animals were scrupulously and attentively cared for so that they were kept in good physical condition. In the typical cases represented in figure 1, the animals, having fully recovered from the final operation, were fastened back-downward on the holder, and that experience plus a single struggle lasting usually for a minute caused a gradual increase of the heart rate which, as the curves of figure 1 show, commonly amounts to about 30 beats per minute (115 records on 23 animals yielded the average figure of 27, with variations between 8 and 48), the maximum being reached in three or four minutes and the recovery requiring eight or nine minutes longer.

Comparison, in figure 1, of the effects of a single struggle, likewise for a minute, when the adrenal nervous connections are intact, reveals at once striking differences. With adrenals innervated the pulse begins to rise a few seconds after the excitement starts, reaches its maximum in about a half-minute, and the original heart rate is not recovered for nearly twenty-five minutes (cf. Cannon and Britton, 1927). Furthermore the quick rise due to secreted adrenin may be as great as 100 beats per minute (in this case 72); whereas the slow rise occurring after adrenal inactivation rarely is higher than 36 beats, and usually is between 20 and 30. Clearly the effect of adrenin fully covers over the effect of the x-factor, and though it may collaborate with adrenin it can be clearly revealed only after the adrenin effect has been removed.

When observations are made on the days soon after denervation of the heart the increases of heart rate, as a consequence of a single struggle, become progressively greater as the days pass. Thus, with cat 18, the rise on the second day was 8 beats; two days later it was 16, again two days and the rise was 20 beats, and twelve days after the operation it was 28. It is impossible to regulate the struggles so that they are uniform, and the result just described might be regarded as due to a rising vigor after the operative disturbance. We have had the impression, however, that there was little difference in the degree of activity and excitement during the period when effects were becoming greater. It was as if the heart were becoming more sensitive to the stimulating agent.

Although excitement with struggle, as in medulliadrenal secretion, is the most effective way of bringing about a faster heart rate by the x-factor, the slight disturbance of the animal caused by transferring it from a comfortable cushion where the basal heart rate was taken, to the holder, would not infrequently increase the rate 6 or 8, but might increase it even 18 beats. We have seen increases from 4 to 10 beats result when the animal merely walked about on the floor for a brief period.

Repeated struggles cause a greater increase of the heart rate than a single one. Thus in cat 266, a single struggle (Feb. 8, 1927) raised the

rate 20 and 24 beats per minute; the next day two struggles, one minute apart, raised the rate 32 beats, and quite characteristically a succession of struggles (five at intervals of one minute) which were induced by turning the holder, raised the rate to maximum 44 beats above the basal level (see also fig. 5). The superposition of the effects of the x-factor doubtless results from later additions built upon the persistence of earlier effects.

As would be surmised, the faster pulse caused by repeated struggles is limited, and, in fact, if they are continued, their effects become less—i.e., the cardiac rate falls in spite of the repetitions of the excitement and activity, as if the x-factor was becoming exhausted or the heart losing its sensitiveness. Obviously no quantitative statement can be made regarding this phenomenon because the struggles themselves are not of uniform intensity; but the failure of the rate to be sustained at the high level, though the disturbances continue, has been so frequently noted that the fact is well established.

The question naturally is asked, why has not the slow, late rise in the rate of the denervated heart been observed before? First, the denervated heart as an indicator of changes in the organism has not been much used. Second, the phenomenon is not clearly manifested *if the animal is fully anesthetized.* Stimulation of the splanchnics or of an afferent nerve in an anesthetized animal, whose heart, adrenals and liver have been denervated, causes discharge of sympathetic impulses (as evidenced by large rises of blood pressure, for example), but it either causes no increase or increases not greater than 2 beats per minute in the cardiac rate (cf. Cannon and Rapport, 1921, who used urethane or ether). Even if the sympathetic fibres in the sciatic nerves or in the lumbar plexus are stimulated while the animal is anesthetized, the effect on the denervated heart, usually alterations of not more than 1 or 2 beats per minute, is negligible (cf. Cannon, Linton and Linton, 1924, who used ether or chloralose). Of course, the comment may be made that these experiments were acute, and that delay after the cardiac denervation might have yielded different results. In an animal which, weeks after the operation, manifested the slow rise due to the x-factor, however, we have noted that under ether anesthesia the heart beat rose (to 172 beats per minute) and that thereafter afferent stimulation caused no further rise. Indeed, even when the sympathetic supply to the heart is present (with vagi cut), and excitement causes great acceleration of the pulse, anesthetizing the animal *fixes* the heart rate at an elevated level and thereafter reflex or direct stimulation of the sympathetics has little or no influence (cf. Cannon and Lewis, 1927). It is not surprising, therefore, that the action of the x-factor has hitherto remained unnoticed.

There is one other characteristic of the slow rise of heart rate to be mentioned—its reduction by any considerable operation and its gradual in-

crease thereafter. Many times our hopes have been excited by indications that an operative procedure had solved the mystery, only to be dashed again by development of the usual effect after a few days had passed. A typical case was that of cat 83. On January 21, three struggles slowly raised the rate 48 beats per minute. That afternoon both superior cervical ganglia were excised. The next day three struggles raised the rate only 12 beats; two days afterwards, however, three struggles caused a rise of 32. From such observations as these it is clear that the search for the source of the x-factor should be made in unanesthetized or lightly anesthetized animals, and that to demonstrate that the source has been removed it must remain removed for considerable time before the fact can be regarded as assured.

THE SEARCH FOR THE UNKNOWN ACCELERATOR. Readers may recall that in a paper describing the method of surgically denervating the heart (Cannon, Lewis and Britton, 1926) there was a section headed by this same caption. At that time we were mystified by a sudden *immediate* increase of heart rate attending emotional excitement, although both stellate ganglia had been removed, vagal connections had been severed, and hepatic and medulliadrenal influences had been excluded. Only after prolonged search was the difficulty resolved by the discovery that accessory accelerator fibres pass to the heart from the upper thoracic sympathetic chains, below the stellates. The effort to find the cause of the *slow* rise of rate after complete denervation of the heart and exclusion of all known accelerating agencies has been quite as demanding of patience and careful consideration of possibilities. Our failures have been significant and are therefore described here.

Not due to rise of arterial pressure. In an earlier paper (Cannon and Rapport, 1921) the evidence was reviewed that the rate of the denervated heart is, within a wide range, independent of changes of arterial pressure. That evidence was based on observations made on the freshly isolated organ, however, and there was a chance that as time passes after the isolation, changes of pressure might become effective. If pressure on the abdominal aorta is used to raise the arterial pressure, it must not interfere with the blood supply to the remaining (denervated) adrenal gland, for asphyxia can stimulate its secretion and thus accelerate the pulse (Zwemer and Newton, 1928). And even after demedullating this gland the pressure may induce slight struggle and thus cause rises in the cardiac rate as high as 20 beats per minute. When that condition is minimized, however, the increments of heart rate due to sudden pressure on the aorta are slight (4 to 8 beats in cats 113 and 265, March 8 to 11) as compared with the effects of struggle (30 beats in cat 113, March 11; 38 beats in cat 265, March 10). It seems probable that even the slight increases of 4 to 8 beats are caused by disturbance of the animal and not to a rise of arterial pressure. When

the sympathetic has been so largely removed that only a small remnant continues active and struggle can induce an increase of pressure only by action of skeletal muscles, it may still induce the slow rise of pulse as much as 28 beats per minute. If now the active remnant (e.g., a few ganglia in the thorax) is removed, struggle no longer markedly accelerates the pulse (see fig. 4). It appears, therefore, that the lasting preparation of the denervated heart, like the fresh preparation, is characterized by indifference to changes of arterial blood pressure, and that the slow rise of rate after excitement and effort is not due to that condition.

Not due to rise of temperature. The well-known influence of rising temperature in causing a faster beat of the isolated heart (cf. Knowlton and Starling, 1912) suggests *that* as possibly the x-factor. Many of the increases of the cardiac rate, however, were far too great to be explained by the heat produced by any struggle; and increases of 8 or 12 beats when animals were first placed on the holder were often not preceded by struggle and therefore cannot be explained by warmed blood. Furthermore, actual measurement of rectal temperature offered no support for the idea. Cat 92, for example, with heart denervated and known accelerators excluded, had, after repeated struggles on the holder during seventeen minutes, a heart rate 30 beats faster than at the start; the rectal temperature meanwhile, instead of rising, had actually fallen 0.2°C. In cat 83, with the same preparatory operations, four struggles speeded up the pulse 48 beats per minute, but raised the temperature only 0.9°C. It is evident that the x-factor cannot be found in temperature increases.

Not due to escape of adrenin from the denervated adrenal medulla or from accessory chromophil tissue, nor to a discharge from the adrenal cortex. Might not manipulation of the animal (turning it on its back and fastening it to the holder), and likewise the struggle, so affect the denervated adrenal that there would be an escape of adrenin which would accelerate the heart? Six cases in which the right adrenal was removed and the left demedullated (as proved by staining and examination after death), and in which the typical slow rises occurred, proved the futility of that suggestion. Furthermore, in four cases, records of the effects of struggle after demedullation showed no noteworthy differences from those taken before. The slow acceleration is, therefore, not explained by adrenin leaking out of the denervated medulla.

Vincent (1910) demonstrated that the accessory chromophil threads and ribbons, found ventral to the aorta in the upper abdomen and supplied with nerve filaments, contain active adrenin. Might not the highly sensitive denervated heart be responsive to adrenin of that origin? This possibility was tested in cats 113 and 286 which, after removal of the ganglia about the roots of the cœliac axis and superior mesenteric artery and clearing away of the tissue ventral to the aorta between the kidneys, many

times exhibited the slow, emotional increases of heart rate, amounting to 20 beats or more. Post-mortem examination of the region for chromophil reaction was negative. Clearly the explanation did not lie there.

The denervated heart is sensitive to adrenin in the dilution of 1 part in 1,400,000,000 parts of blood (Anrep and Daly, 1925). This extreme sensitiveness would reveal any, even slight, escape of adrenin from the denervated medulla. But Hartman and Hartman (1923) have reported finding adrenin in small amounts also in the adrenal cortex. That there is evidence of no escape of adrenin from the denervated medulla, where adrenin is abundant, indicates that there is likewise no escape of adrenin from the denervated cortex, where it is scarce. In addition, the same rises occurred in four animals after the adrenal which remained had been subjected to the great disturbance of its structure that is involved in demedullation. The adrenal cortex, therefore, does not explain the x-factor.

Not due to the cardio-accelerator substance from the liver. The only other known humoral cardiac accelerator besides adrenin is that produced when liver nerves are stimulated (Cannon and Uridil, 1921). Of course, it was not impossible that the x-factor and the hepatic accelerator were the same. The rapid action of the agent given off by the liver, however,—an action almost as rapid as adrenin itself (cf. Cannon and Griffith, 1922) was not consistent with that idea. Further, the substance produced by the liver was much more potent when the animal was digesting meat. We have recorded the effects of struggle in many animals while they were fasting and again during protein digestion and have observed no noteworthy difference in the slow rises of heart rate. Again, the hepatic accelerator was not interfered with by anesthesia; the x-factor was. And finally in six of our animals not only were the nerves to the liver severed, but the medulla of the remaining adrenal was removed. All possibility of sympathetic or medulliadrenal control of the liver was thus excluded, and yet the slow increase of heart rate persisted. We infer, therefore, that the phenomenon is not explained by action of the hepatic cardio-accelerator.

Not due to a substance produced by the pancreas, gastro-intestinal mucosa, or semilunar ganglia. All known agencies making the heart beat faster had now been ruled out. And yet some substance, delivered to the heart by the blood, was regularly causing the faster beat whenever the animal engaged in excited struggle. The sympathetic system is characteristically involved in activity under these circumstances. Might not the pancreas or the mucosa of the stomach and intestines, stimulated by the sympathetics, be the source of the substance? Or might not there be chromophil cells in the semilunar ganglia which could discharge and accelerate the pulse? The answer to these questions we found in four animals in which, after denervation of the pancreas, the stomach and the small intestine, by excision of the upper abdominal sympathetic chains and of the semilunar

ganglia as well, the typical effects of the x-factor were still clearly manifest. Furthermore, in one animal the slow rise continued although in addition to the operation just mentioned, the remaining left vagus was sectioned in the neck. We conclude therefore, that the questions asked above must be answered in the negative.

Not due to discharge from the pituitary body or the gonads. The observation of Dandy (1913) that numerous nerve fibres enter the pituitary body from the nerve plexus on the blood vessels which surround the organ, the testimony of Weed, Jacobson and Cushing (1913) that stimulation of

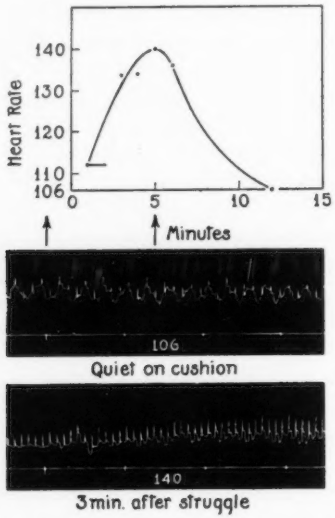

Fig. 2. Graph showing the slow increase and decrease of pulse rate in a castrated male cat (286), after denervation of the heart, exclusion of all abdominal sympathetic influences and removal of the hypophysis. See figure 1 for arrangement of the graph. The original records were taken at periods indicated by the arrows.

the superior cervical ganglion produces effects characteristic of injected pituitrin, and the evidence that emotional excitement evokes a discharge from the posterior lobe (Hoff and Wermer, 1928) made us suspect that organ. That it could not be involved by way of the sympathetic system, however, was proved by observing the usual slow rise in four animals after cardiac denervation and hepatic and medulliadrenal exclusion plus extirpation of the cervical sympathetic strands with their inferior and superior ganglia. The demonstration by Greving (1925) and Pines (1925) of an abundant nerve supply directly from the base of the brain to the posterior lobe made possible an influence coming to the pituitary by that course,

and thereby an effect on an endocrine organ which might slowly accelerate the pulse (cf. Bacq and Dworkin, 1930). Cat 286, however, after denervation of the heart, total exclusion of both abdominal sympathetic chains, section of the left splanchnics, removal of the right adrenal and sucking out of the medulla of the left, cutting of the nerves on the duodenohepatic artery and later those on the coeliac axis and the superior mesenteric arteries, and later still the right splanchnics—of course, in a succession of stages—underwent (June 21, 1927) a hypophysectomy. The animal, a *castrated male,* lived in the laboratory in excellent physical condition for fifteen months, when sacrificed to determine the thoroughness of the operative procedures. After the hypophysectomy, excitement and struggle still induced the typical slow rise of heart rate (see fig. 2). One other castrated male (cat 302) likewise manifested the phenomenon. Also removal of the ovaries from a female (cat 300) did not affect the slow rise. Again the x-factor was left unexplained.

Not due to activity of the thyroids or parathyroids. Certainly, so far as the thyroid and parathyroid glands are concerned, the possibility of sympathetic stimulation was abolished in cases of removal of the cervical sympathetic strands, and probably it was abolished in all cases, because cardiac denervation involved extirpation of the stellate ganglia through which pass the preganglionic fibres distributed to the cervical ganglia. Under both conditions the x-factor was fully effective. Of course, the objection may be offered that the innervation of these glands may arise from cranial autonomic sources. We have ruled out that possibility, however, with respect to the thyroids, by removing them and observing the same typical slow rise of heart rate when thereafter the animal struggled. We did not remove two parathyroids in these operations, but the taking out of the other two and the disturbance of the remnants incident to the operation had no influence. Furthermore, in cat 82, to be described more in detail later, transection of the spinal cord, at the seventh thoracic vertebra, was followed the next day by several struggles involving the fore part of the animal (in which the pituitary body, the left thyroid lobe, and the two left parathyroids remained undisturbed); these struggles did not cause a rise of more than 8 beats per minute, although before the operation single brief struggles had increased the pulse 30 and 34 beats. From all these observations, we conclude that the x-factor does not originate in the thyroids or parathyroids.

Not due to metabolites liberated from skeletal muscle. The association of the slow rise of rate of the denervated heart with struggle suggested that possibly some substance given off by skeletal muscle was the active agent. To be sure, that possibility had been excluded in *anesthetized* animals by Cannon, Linton and Linton (1924), but might it not be true in unanesthetized animals? That the activity of skeletal muscles was not an essential

source of the x-factor, was shown by the faster pulse as the animal was transferred from the comfortable place on the cushion to the holder. Commonly this move was not accompanied by any struggle of the animal and yet the cardiac rate would increase 6 or 8 beats or more. That metabolites from skeletal muscle do not actually play an important rôle, was proved by observations on cat 113. This animal had the heart denervated, upper abdominal sympathectomy, the right and left splanchnics sectioned, right adrenal excised and left adrenal medulla sucked out. Vigorous struggle for one minute (March 10 and 11, 1927) caused slow increases of the heart rate 20 and 28 beats above the basal. Metal band electrodes, covered with gauze wet with brine, were applied to the shaved skin, one on each lower hind leg. Stimulation which caused tetanic spasms of the leg muscles, was followed either by no change of pulse or by an initial fall (8 to 12 beats) with later partial or full return to the original rate and, in two cases, by a rise of 4 beats above that level. As under anesthesia so here, active muscular contractions did not induce a faster heart rate. They left the x-factor still a mystery.

SUGGESTIVE EVIDENCE AS TO THE SOURCE OF THE MYSTERIOUS CARDIO-ACCELERATOR. We looked for the clue to the slow cardio-acceleration in products of endocrine glands because, with the blood stream the only connection between the heart and the rest of the organism, some chemical change in the blood must account for the phenomenon and such a change could naturally be caused by an internal secretion. When, one after another, the endocrine glands were excluded from action and yet excitement continued to make the heart beat more rapidly, we were baffled and almost in despair of finding an explanation. The association of the curious effect with emotional disturbance and the conviction that the sympathetic system must be implicated in disturbance of that type led us to attempt to learn whether the x-factor might arise from some previously unrecognized source in the fore part or the hind part of the animal. The experiment was tried on cat 82. On December 9, 1926, the heart had been denervated, the thoracic sympathetic chains removed as far back as the ninth ribs, the right adrenal excised, and the nervous connections of the left adrenal and the liver cut. The right stellate ganglion was left in place but its cardiac connections were severed. Later the right thyroid and parathyroid as well as the superior cervical ganglia were extirpated. After such exclusion of endocrine factors a slight struggle, January 4, 1927, raised the heart rate 30 beats (from 124 to 154 per minute). On January 5, the spinal cord was transected with surgical care at the seventh thoracic vertebra. The next day struggles, one of which was vigorous, caused the maximal rise of the pulse for the day—8 beats (from 82 to 90). This was the sort of struggle which, with the spinal cord intact, would result in a marked acceleration. The fact that no considerable acceleration occurred

proved that the source of the x-factor was not anterior to the region of transection of the cord, i.e., in that part, subject to the cerebrum, where excitement and effort occurred.

In order to test the hind part of the animal, we inserted needle electrodes, insulated by shellac except at the tip, one in the eighth thoracic intervertebral opening and the other in an opening between the lower lumbar vertebrae, for stimulation of the isolated part of the cord. Strong stimulation caused extension of the legs, erection of the tail hairs, increases of the pulse by 16 beats twice, and by 32 beats in a third trial. A thermometer in the rectum showed a rise of only 0.35°C. There was no indication of disturbance of the fore part of the animal by the occurrences in the hind part, except the faster heart rate which increased slowly to a maximum and slowly returned again to the original level.

On January 7, we repeated these experiments, except that the anterior electrode was placed first in the tenth thoracic intervertebral opening and later in the first lumbar opening. When the electrode was in this posterior position the spinal cord was divided again at the twelfth intervertebral opening in order to give the greatest possible assurance that there was no spread of the stimulating current forward. In the first position of the electrodes the stimulation, at 11:36, with the secondary of the Harvard induction coil at 7 cm., caused extensor spasm of the hind legs, no effect on the tail, and an increase of only 4 beats per minute in the heart rate. At 11:50 the secondary was moved over the primary to the 4 cm. mark and stimulation for 30 seconds caused again extensor spasm, but this time the tail hairs stood erect and the pulse rate rose from 90 to 128, the maximum being reached at 11:52. There was no sign of any effect in the fore part of the animal—for example, no change of respiration.

The left adrenal, though denervated, was still in place. To make certain that it was not secreting adrenin under the circumstances, and thus accelerating the pulse, it was tied off by a mass ligature at 12:35. Now stimulation as at 11:50 caused erection of the tail hairs, extensor spasm and, at the end of two minutes, an increase of 26 beats. Another stimulation caused similarly an increase of 22 beats. Then the isolated portion of the spinal cord was severed, as stated above, and the anterior electrodes were moved to the lumbar region. Stimulation for 30 seconds with the secondary at 7 cm., raised the heart rate slowly 12 beats, and with the coil at 4 cm. the same duration of the stimulus raised the rate 26 beats in two and a half minutes. The tail was bushy and there was extensor spasm in the hind legs, but no spreading whatever beyond this region. The rectal temperature did not change. Steps in the original record are shown in figure 3.

These observations roused much interest and speculation. Clearly some

agency from the hind part of the animal was entering the blood stream and causing the heart to beat faster. What it was we did not surmise. It probably was liberated by means of sympathetic impulses; and since we were trying to find conditions which would leave the heart unaffected by struggle, we resolved to remove the abdominal sympathetic strands from diaphragm to pelvis and see if the heart rate would then remain "flat." This was done, and to our great surprise the slow rise of heart rate still continued!

The impasse presented by the bewildering situation just mentioned was first satisfactorily surmounted by an operation on cat 303. This animal, which on August 10, 1927, had had a cardiac denervation with thoracic

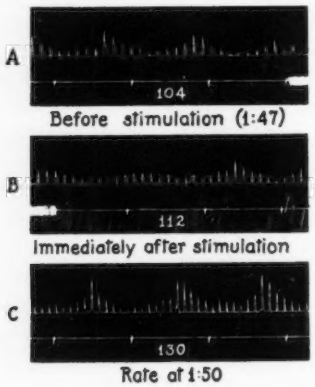

Fig. 3. Cat 82. Original records showing the slow rise and fall of the heart rate when, after severance of the spinal cord in two places in the thoracic region, the lumbar cord was stimulated. A, just before stimulation started, at 1:47, heart rate 104 beats per minute; B, at end of stimulation for 30 seconds, heart rate 112; C, at 1:50, heart rate 130. Struggle by the fore part of the animal was without noteworthy effect on the heart rate.

sympathectomy as low as the eighth rib, a bilateral abdominal sympathectomy covering connections with the spinal cord, a left splanchnic section, a right adrenalectomy and the liver nerves cut, repeatedly showed, between August 31 and September 19, slow increases of the heart rate after struggle. On September 20 the lower chest was opened and the remnants of the thoracic chains from the tenth rib to the diaphragm were removed. Our gratification can be imagined when, after full recovery of vigor, the animal was tested and the cardiac rate was found to be "flat," and when, furthermore, week after week for about two and a half months it remained so (see fig. 4). Repeated struggles which strikingly augmented the heart rate before sympathectomy had no such effect after the operation (see

fig. 5). The same final operation, removing the lower thoracic remnants, placed cats 287 and 291 in the same condition as cat 303. They did not remain "flat" as long as 303, however, because their hearts had been de-

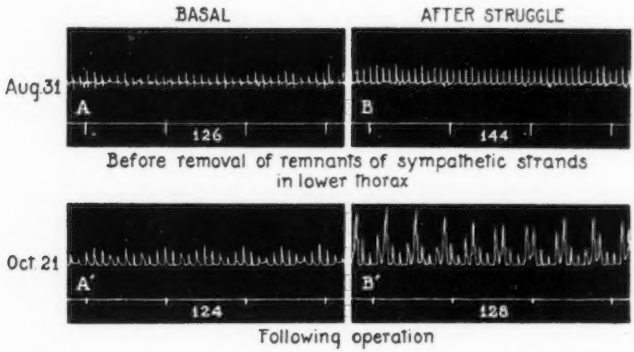

Fig. 4. Cat 303. Original records of the basal rate of the denervated heart (A, A^1) and the rate three minutes after a vigorous struggle, (B, B^1). Records A and B were taken before removal of remnants of the sympathetic strands in the lower thorax, and A^1 and B^1 were taken four and a half weeks after that operation.

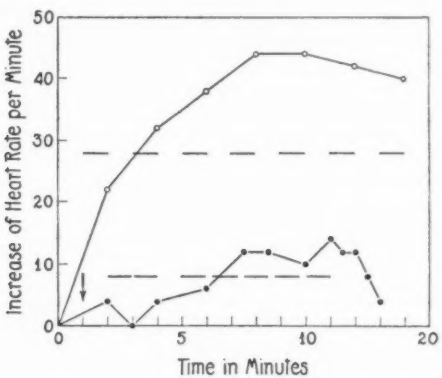

Fig. 5. Graphs showing the effect of seven struggles, at intervals of one minute, on cat 265 (Mar. 10, 1927), upper line, and cat 303 (Oct. 11, 1927), lower line. In each animal the heart was denervated, the adrenals inactivated, and the liver nerves cut. In cat 303, however, all sympathetic connections with the central nervous system had been severed.

nervated for a longer time and consequently nervous connections between the vagi and the heart became reëstablished sooner. Indeed, the difficulties encountered in animals which long survived cardiac denervation were

frequently complicated by regrowth of vagal fibres, as shown by sharp changes in heart rate which disappeared under the influence of atropine. The word "flat" should not be taken as indicating strict fixity of the heart rate. It varied from day to day, and after struggle it rose commonly 4 or 6 beats, but even when violent and prolonged muscular effort was exhibited it did not rise more than 10 or 12 beats—a rise which could be accounted for by the development of heat. By proper section of the vagal branches to the heart and complete removal of the sympathetic chains from stellate ganglia to the pelvis in one piece, "flat" preparations could now be made. This fact led to that mode of performing sympathectomy. The animals thus operated on were new organisms in the history of physiology—vertebrates without sympathetic control of the viscera—and they became objects of interest in themselves (see Cannon, Newton, Bright, Menkin and Moore, 1929) and they also served for interesting experiments on the effects of different foods on the isolated heart (Moore, 1929). For these reasons enquiry into what caused the mysterious rise of heart rate when the lower thoracic sympathetic was still in place was not immediately continued. Was there a reticulate plexus in the aorta which slowly conducted impulses up to the heart from the lower thorax? Were there connections between the lower thoracic ganglia and abdominal viscera, that had been overlooked? These questions arose, but, as other engagements required two of us to leave the work, the questions were set aside—and the mystery of the x-factor remained unsolved.

SUMMARY

After complete denervation of the heart and exclusion of all known humoral cardio-accelerator agencies a momentary excitement and struggle will cause, in cats, a slow increase of the cardiac rate, reaching a maximum in about three minutes and then slowly subsiding (see fig. 1).

Repeated struggles cause summation of effects on the heart rate (see fig. 5).

Full anesthesia (with ether, urethane or chloralose) greatly reduces and may abolish the phenomenon, i.e., stimulation of muscles and the sympathetic system under anesthesia does not produce the typical effect on the heart rate.

Experiments are described which show that the phenomenon is not due to rise of arterial blood pressure, increase of temperature, escape of adrenin from the denervated adrenal medulla or cortex, or from the liver, nor due to substances produced by the pancreas, gastro-intestinal mucosa, semilunar ganglia, pituitary body, male gonads (see fig. 2), thyroids, parathyroids or active skeletal muscles.

Transection of the thoracic spinal cord in the prepared cat leaves the fore part of the animal with the denervated heart, and the hind part with

the denervated adrenal and liver, but with the lower abdominal sympathetic strands still intact. Struggle by the fore part of the animal is without effect; stimulation of the spinal cord in the lumbar region, causing muscular spasm and erection of the tail hairs, is followed by the typical slow rise and fall in the rate of the denervated heart (see fig. 3). Obviously a substance is given off into the blood-stream by the hind part of the animal that affects the heart in the fore part.

If all parts of the sympathetic system are disconnected from the spinal cord, except short ganglionated strands in the lower thorax, struggle causes the usual cardiac acceleration; but after the remnants of the system are excised, struggle no longer has that effect. This condition may last for many weeks (see figs. 4 and 5).

The solution of the mystery of slow acceleration of the denervated heart after excitement and effort, when all known accelerators are excluded, is described in the following paper.

BIBLIOGRAPHY

ANREP, G. V. AND I. DEB. DALY. 1925. Proc. Roy. Soc., London, B. xcvii, 454.

BACQ, Z. M. AND S. DWORKIN. 1930. This Journal, xciii, 629.

CANNON, W. B. AND S. W. BRITTON. 1927. Ibid., lxxix, 433.

CANNON, W. B. AND F. R. GRIFFITH. 1922. Ibid., lx, 544.

CANNON, W. B. AND J. T. LEWIS. 1927. Ibid., lxxxii, 67.

CANNON, W. B., J. T. LEWIS AND S. W. BRITTON. 1926. Ibid., lxxvii, 326.

CANNON, W. B., J. R. LINTON AND R. R. LINTON. 1924. Ibid., lxxi, 153.

CANNON, W. B., H. F. NEWTON, E. M. BRIGHT, V. MENKIN AND R. M. MOORE. 1929. Ibid., lxxxix, 84.

CANNON, W. B., A. QUERIDO, S. W. BRITTON AND E. M. BRIGHT. 1927. Ibid., lxxix, 465.

CANNON, W. B. AND D. RAPPORT. 1921. Ibid., lviii, 308.

CANNON, W. B. AND J. E. URIDIL. 1921. Ibid., lviii, 353.

DANDY, W. E. 1913. Amer. Journ. Anat., xv, 333.

GREVING, R. 1925. Zeitschr. f. Anat. u. Entwicklungsges., lxxvii, 249.

HARTMAN, F. A. AND W. E. HARTMAN. 1923. This Journal, lxv, 623.

HOFF, H. AND P. WERMER. 1928. Arch. f. exper. Path. u. Pharm., cxxxiii, 97.

KNOWLTON, F. P. AND E. H. STARLING. 1912. Journ. Physiol., xliv, 206.

MOORE, R. M. 1929. This Journal, lxxxix, 515.

PINES, J. L. 1925. Zeitschr. f. d. gesammt. Neurol. u. Psych., c, 123.

VINCENT, S. 1910. Proc. Roy. Soc., London, B lxxxii, 502.

WEED, L. H., H. CUSHING AND C. JACOBSON. 1913. Johns Hopkins Hosp. Bull., xxiv, 40.

ZWEMER, R. L. AND H. F. NEWTON. 1928. This Journal, lxxxv, 507.

STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE ORGANS

XXVI. A Hormone Produced by Sympathetic Action on Smooth Muscle

W. B. CANNON and Z. M. BACQ[1]

with the assistance of R. M. Moore[2]

From the Laboratories of Physiology in the Harvard Medical School

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The only positive evidence obtained by Newton, Zwemer and Cannon (1931) to account for the mysterious emotional acceleration of the denervated heart observed by them after they had excluded all known humoral accelerators, came from one case in which the isolated lumbar cord was stimulated. The heart was accelerated, although all nervous connections between the hind part and the fore part of the animal had been destroyed. Unfortunately the experiment was not wholly satisfactory, for the stimulation caused strong contraction of the striated muscle of the legs, as well as of the smooth muscle of the tail, and there might have been effects on other organs. Under the circumstances it was hazardous to draw conclusions. Nevertheless, the absence of a faster heart rate when the stimulus did not cause the tail hairs to rise, and its regular appearance when the hairs stood, were highly suggestive facts. They were supported by the observation that removal of the last remnants of the sympathetic chains which were still connected with the spinal cord, those of the lower thorax, left the heart practically unaffected by emotional excitement—a "flat" preparation. To be sure, questions arose as to a possible influence of fibres from the lower thoracic ganglia on organs in the abdomen and a possible slow conduction through a nerve plexus in the wall of the aorta; but by associating the disappearance of the acceleration on completing the sympathectomy with the appearance of the acceleration when the tail hairs were erected, the simple inference might reasonably be drawn that the lower thoracic ganglia, innervating smooth muscles in the skin of the back, had the same effect as the sympathetic supply to the tail, i.e., evoked from them a substance capable of making the heart beat

[1] Fellow of the C. R. B. Educational Foundation.

[2] Medical Fellow of the National Research Council.

faster. The striking disappearance of the emotional tachycardia when these last functioning parts of the sympathetic system were extirpated would thus be readily explained. In order to make this explanation valid, however, the actual production of a cardio-accelerating substance from smooth muscle alone must be demonstrated.

INITIAL EXPERIMENTS. The foregoing argument and suggestion for further tests led one of us (C) to undertake with R. M. Moore, in the autumn of 1928, some experiments directed towards the solution of the problem. Eight animals (cats) were used, six under light ether anesthesia, one under amytal, and one with the brain destroyed and with the spinal cord pithed to the mid-thorax. All were acute experiments. In all except one the heart was denervated by section of the vagi in the neck and removal of the upper thoracic sympathetic chains, each adrenal was tied off by mass ligation, and the hepatic nerves were cut. The abdominal sympathetic chains over the sacral promontory were exposed, tied, cut, and laid on electrodes. Stimulations of the peripheral ends of the cut chains, for periods varying from 20 to 60 seconds, resulted (Oct. 27, 1928), first, in erection of the tail hairs and an increase of heart rate of 6 beats per minute about one and a half minutes after the stimulation; second, in an increase of 14 beats after an interval of about two minutes; third, in an increase of 6 beats after about three minutes; and thereafter no certain effect. This result looked promising, but not good enough. We thought that amytal might provide a better mode of anesthesia, but under its influence no effects were obtained. We then returned to light ether, and repeated our first experiment. Stimulations (Oct. 31, 1928) for 60 seconds caused first a small rise of 9 beats, then a rise of 18 and later one of 22 beats per minute. The peak was reached in each instance about a minute and a half after the end of the stimulation. It was interesting to note that stimulation was accompanied by a gradual increase of arterial pressure which reached its maximum—in two instances a rise of 20 mm. of mercury —at the end of the minute during which the stimulus was applied, and which remained high thereafter, only slowly returning to the original level as the heart rate did, five or six minutes after the start. On November 2 we had another slight success by use of the same method, a single rise of 14 beats; but an attempt to repeat it the next day resulted in a single rise of 6 beats and then no change. The method was evidently unreliable because, unlike emotional stimulation, it was not constant. We thought that the variability might be due to deep anesthesia during the operative preparation. We therefore tried the experiment in an animal pithed through the orbit to the mid-thorax, a procedure which at once denervated the heart and adrenals and also obviated the need for continued anesthesia, though it required, of course, artificial respiration. Stimulation of the sympathetic supply to the tail, however, was wholly without

effect—the heart rate, 160 beats per minute, did not change. A return to light ether, November 7 and 10, resulted in negligible rises of only 4 and 6 beats. Other interests meanwhile had developed and consequently these experiments, with some promising and other very disappointing results, were not continued.

In the autumn of 1929 the present authors renewed the effort to discover the nature of the slow rise of heart rate seen not only after emotional excitement but seen also occasionally, as shown by the first series of experiments, on stimulating smooth muscle through sympathetic fibres. Fourteen new experiments were performed. In the main they were directed against the rather long operative preparation required in the first experiments, though six were practically repetitions of them—two under amytal, two under ether, and one under magnesium sulphate followed by calcium chloride (see Meltzer and Auer, 1908), being negative, and one under light ether yielding rises of heart rate of 12 and 10 beats in two records. In the remaining eight cases, the heart was partially denervated (by extirpating the upper thoracic sympathetic strands), one adrenal was removed and the other denervated, and in some instances the liver nerves were severed, in a preliminary surgical operation. On the day of the experiment the animal was etherized, the vagi were cut in the neck to complete the cardiac denervation, the common carotid arteries were tied, the cerebral hemispheres ablated, and the lower abdominal sympathetic strands bared and laid on shielded electrodes. In some instances the sympathetic strands were bared on the dorsal side of the vessels at the base of the tail and were stimulated there by applying bipolar electrodes. After the decerebration the ether was discontinued. Of these eight experiments two were negative —one associated with low blood pressure, the other with no erection of the tail hairs. Two had a single rise of 4 beats—one of these associated with a very slight erection of the tail hairs, the other with a fast pulse (in a digesting animal). The failure of the tail hairs to stand was probably due to injury of the sympathetic strands when they were set on the electrodes, which were unduly large and unwieldy. The four other experiments were positive. One yielded repeated increases of heart rate; not larger than 6 beats per minute, to be sure, but regular and definite. The second had increases of 14 and 6 beats. In the third (Oct. 18, 1929) the heart rate rose slowly from a minute of stimulation and in two minutes was at its peak, 18 beats above the basal. Six minutes later it had gradually returned. A second stimulation had a similar effect, but the rise was only 12 beats. On each occasion the tail hairs stood.

The results obtained in the fourth case (cat 269), in which the heart rate was recorded in a blood pressure curve, are partially illustrated in figure 1. A first stimulation had been without effect—not uncommonly, in our experience, the first effect may be slight, or smaller than later effects

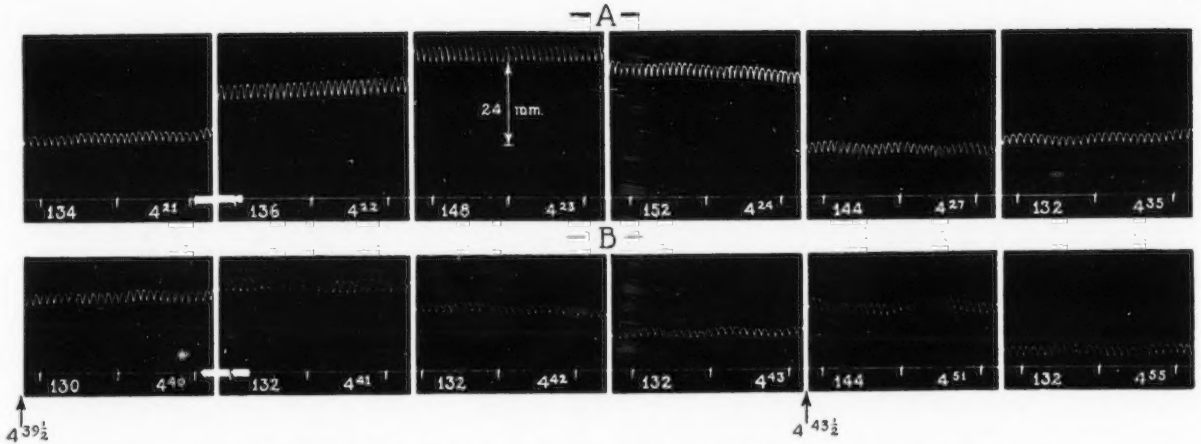

Fig. 1. Excerpts from the record of cat 269, an animal with heart denervated, adrenals inactivated, carotids tied and cerebrum ablated. Electrodes placed on lower abdominal sympathetic strands which were isolated from the spinal cord. A loop of strong thread was passed around the aorta and inferior vena cava above the iliac branches. A cannula for the blood pressure record was inserted into the right carotid artery.

A. Stimulation for one minute, between 4:21 and 4:22, was followed by the changes of blood pressure and heart rate seen in the records at the times indicated.

B. Stimulation of the same strength as in record A, for one minute, between 4:40 and 4:41, while the blood flow in the hind part of the animal was checked by closure of the aorta and inferior cava, between 4:39½ and 4:43½, as shown by the arrows. Note that at 4:43, corresponding to 4:24 in record A, the heart rate had risen only 2 beats, and the blood pressure had fallen. At 4:48, 4½ minutes after removal of the block, the rate had risen 12 beats; at 4:51, when 14 beats above the basal, the rate began to fall, and four minutes later had returned nearly to its former level. Record reduced one-half.

(see fig. 2 *a*). In response to the second, slightly stronger stimulation (one minute, no spread of current, tail hairs standing) the heart rate gradually rose 18 beats two minutes after the stimulus was stopped and then gradually fell, during the next eight minutes, to the original level. The blood pressure likewise went up slowly 24 mm. of mercury, but reached its highest point about a minute sooner than the heart rate did (see fig. 1). A second stimulation was started just after a ligature was tightened around the inferior vena cava and the aorta, directly anterior to the iliac branches. Two minutes after the stimulation (for one minute, and of the same strength as before) had ceased, the heart rate had increased only two beats instead of 18; the block on the blood flow was then lifted and blood allowed to enter the region where, as proved by erection of the tail hairs, the smooth muscle had been stimulated. Slowly the heart rate began to rise, and at the end of four minutes had reached a plateau, 12 to 14 beats above the basal, where it remained for about three minutes and then slowly fell, during four minutes, to the original level. The blood pressure, which did not rise, but instead fell sharply when the block on the blood flow was removed, slowly rose and fell with the heart rate. It is clear that a check on the circulation in the hind part of the animal hindered the carriage of some material from that region to the heart. The experiment showed also that the positive effect was not due to electric stimulation spreading to the fore part of the animal, and that the cardio-accelerator substance has a pressor influence on the blood pressure.

The few positive experiments in this series were not reassuring as to the method. We wished to find a means of demonstrating acceleration of the heart, on stimulating a part of the sympathetic system, that would be as reliable as emotional stimulation. The necessary absence of one of us (C.) for ten months interrupted the work. It was undertaken again in September, 1930.

Final experiments. In order to obtain assuredly consistent results we resolved to produce conditions which would be, as nearly as possible, those under which emotional acceleration of the denervated heart occurred, but which, without anesthesia, would permit local electric stimulation of sympathetic fibres distributed to smooth muscle. Accordingly, by surgical methods, the heart was denervated, except the left vagal connections, in the usual way (i.e., by removing the thoracic sympathetic strands from the stellate ganglia to the seventh or eighth ribs, severing the right vagus nerve below the recurrent laryngeal branch and also the common cardiac nerve); the right adrenal was removed, the left denervated (by section of the left splanchnics and extirpation of the left upper abdominal sympathetic strand); and the liver nerves were cut. After a week or ten days recovery from the operation was complete. On the day of the acute experiment the animal was quickly anesthetized with ether, the left vagus

nerve was severed in the neck (thus completing the cardiac denervation), and the spinal cord was transected at the base of the seventh thoracic vertebra. The severance of the spinal cord at this level, just above the sympathetic strands in the lower thorax, effectively disconnected from the brain all remnants of the sympathetic system previously under central control. In other words, the result was a heart unaffected by any struggle or disturbance by the fore part of the animal. We cannot emphasize too strongly the *routine regularity* of the heart rate in such preparations. In figure 2 are indicated the very slight variations of the basal level during more than three and a half hours of experimenting—and even these variations, we are sure, are due to our failure to wait for complete return of the disturbed heart to its resting rate before it was disturbed again.

The procedure of severing the left vagus nerve and the spinal cord takes so little time that the animal soon eliminates the ether used for anesthesia. In the afternoon, five or six hours after the morning operation, the experimental manoeuvres were carried out on the hind part of the animal, which, separated from the brain, was, of course, quite without feeling. For excitation of the pilomotor muscles the sympathetic was laid bare at the root of the tail, as previously mentioned (see p. 394), or on the dorsal side of the caudal end of the aorta and inferior vena cava between the sixth and seventh lumbar ganglia (Langley, 1894)—a location for the shielded electrodes which was made more approachable by tying and cutting the last lumbar artery and vein on one side—; and for effects on the smooth muscle of the intestines the sympathetic fibres accompanying the mesenteric arteries were isolated. Invariably the central connections of the fibres were interrupted by crushing or cutting them. Of course, stimulation of any of these nerves involves the smooth muscle of the blood vessels, and if that fact is not mentioned it is understood. Stimulation of the lower abdominal chain affects also the sweat glands of the foot, but the result is not different when the fibres at the root of the tail are stimulated. We therefore felt justified in using the convenient region between the sixth and seventh lumbar ganglia. By use of this method the effects of electric stimulation of sympathetic strands became quite as regular and reliable as the effects of emotional stimulation—the heart rate, which was usually recorded by a tambour set against the thoracic wall (see Cannon, Lewis and Britton, 1926), was increased in the typical manner. In numerous experiments performed according to this technique we have never failed to obtain the characteristic cardiac acceleration.

The typical rise of heart rate on stimulating the sympathetic supply to smooth muscle. In animals prepared as described above and not fed since the previous afternoon, the rate of the denervated heart is satisfactorily slow—usually about 140 beats per minute. Stimulation of isolated lower abdominal sympathetic strands for 30 seconds, from a standard position

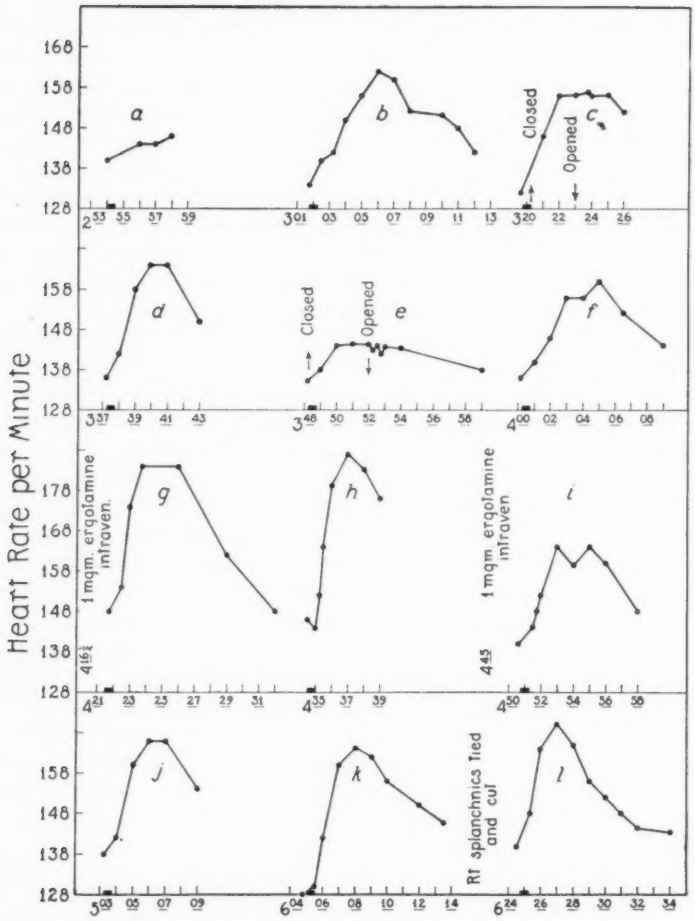

Fig. 2. Experiment, October 15, 1930. Increases of the rate of the denervated heart after repeatedly stimulating, at the lines indicated, the sympathetic supply to the smooth muscle of the tail (except *l*) 30 seconds in each instance, with the secondary coil fixed at 8 cm. At *c*, the blood flow was blocked (as shown by arrows) for nearly 3 minutes *after* the stimulation period. At *e*, the blood flow was blocked *during* the stimulation period and for nearly 3.5 minutes thereafter. At 4:16½ 1 mgm. ergotamine tartrate injected intravenously. At 4:45 the dose was repeated. The stimulation which caused increases of the heart rate at *j* and *k* was not accompanied by erection of the tail hairs. The increase at *l* followed stimulation of the isolated splanchnic nerves for 30 seconds.

of the secondary coil of a Harvard inductorium at 8 cm., without any spread of current, caused an increase of heart rate averaging slightly less than 13 beats per minute in 22 records on nine animals. The rise is characteristically slow, reaching its maximum between 1.5 and 3.5 minutes after the stimulation ceases; in eleven instances of high rises of the rate the peak was reached in about 2.25 minutes. The return to the resting rate is much slower, requiring not uncommonly 7 or 8 minutes for its completion (see fig. 2).

It is not necessary to confine the stimulation to the sympathetic strands at the brim of the pelvis. We have stimulated sympathetic fibres at the root of the tail, after the exposure already described (see p. 394), and have obtained typical accelerations of the heart rate. Also we have obtained the same effect on stimulating the peripheral ends of the cut splanchnic nerves on the side from which the adrenal had been removed, in an animal with hepatic nerves severed (see fig. 2, *l*). The rise, an increase of 30 beats, occurred slowly and became maximal nearly two minutes after the stimulation ceased. About seven minutes later the initial rate had been approximately restored. Furthermore, stimulation of the sympathetic fibres passing out to smooth muscle of the uterus and bladder or to the colon from the isolated inferior mesenteric ganglion causes a typical increase of the heart rate (see fig. 3).

After obtaining these consistent results we thought that we might save the time required for recovery from the surgical preparation by exactly repeating the procedure in a morning operation and using the animal, with spinal cord severed, in an afternoon experiment. Accordingly between 10:00 and 10:55 a.m., under ether, the heart was denervated, the adrenals and the liver were inactivated and the spinal cord was severed. About 4:00 p.m. the lower abdominal sympathetic strands were exposed and placed on shielded electrodes. The usual stimulation for 30 seconds caused the pulse to quicken 10 beats per minute But all attempts to repeat the phenomenon were quite futile (cf. p. 393). Although the tail hairs were erected, fully as much by the later stimulations as they were by the first, there was no change in the heart rate. We were compelled, therefore, to return to our routine method.

The effect of checking the blood flow in the region stimulated. If the blood flow through the hind end of the animal is hindered by closing the aorta and inferior cava above the iliac branches *before* applying the stimulus, the response of the heart is markedly altered. In an experiment of this type, October 15 (see fig. 2 *e*), the increase of the cardiac rate two and a half minutes after the end of stimulation was slightly more than 8 beats per minute instead of 24 or more as it had been twelve minutes before (fig. 2 *d*) or was thirteen minutes later (fig. 2 *f*), as a result of the same stimulus. This inclusive block was present for four minutes. When it was removed

the pulse was not further quickened, but the slightly faster rate was prolonged and only gradually disappeared. When the block was applied at the end of stimulation instead of at the start the initial rise began, but did not continue to the usual height, and became a plateau, higher, to be sure, than that seen in the inclusive block, but otherwise like it (see fig. 2 *c*). After recovery from the inclusive block the standard stimulus was somewhat less effective than it had been before—the increase of heart rate was 24 beats per minute instead of 28—and the rise was slightly less abrupt (see fig. 2 *f*).

In our experiments on shutting off the circulation in the hind legs and tail the results just described have been typical. We have not had a repetition of the complete absence of the acceleration until the block was removed, like that illustrated in figure 1. In consideration of the collateral vascular connections through the spinal canal and in subcutaneous areas the reduction of the effect seems to us more to be expected than its abolition.

The effect of ergotoxine and ergotamine. These drugs are known to block the positive action of sympathetic impulses and adrenin on smooth muscles and glands. They also have a similar effect in the heart, but a relatively large dose is required as compared with that affecting, for example, the smooth muscle of the blood vessels (Dale, 1906). It seemed possible, therefore, that by use of these drugs we might obtain interesting information regarding the relations of the smooth muscle which we were stimulating and the responding cardiac muscle. At first we used ergotoxine (Burroughs, Wellcome) and later ergotamine (Sandoz). The results occasioned by the two preparations were not observably different.

The first and most striking influence of ergotoxine or ergotamine is a marked augmentation of the efficiency of a uniformly repeated stimulation of smooth muscle as a means of accelerating the heart beats. For example, on October 15, two stimulations of the pilomotor muscles of the tail had produced maximal increases of the heart rate of 28 beats per minute (see fig. 2, *b* and *d*). An intravenous injection of ergotamine, and repetitions of the same stimulus as before made the rise 36 and 43 beats (fig. 2, *g* and *h*). There was slight erection of the hairs. Later, however, when stimulation caused no erection of the hairs, the rise was 28 and 36 beats (fig. 2, *j* and *k*). This observation was confirmed by other experiments. On October 7, increases of 9, 10 and 11 beats were raised to 16 and 17 after ergotoxine, with no change of stimulation intensity; and on October 27, when the electrodes were applied to the postganglionic fibres on the inferior mesenteric artery, ergotoxine changed the acceleration from 12 to 32 beats per minute (see fig. 3).

The greater maximal increases in heart rate after ergotoxine were not attended by corresponding increases of the periods during which the rate was rising and falling back again. In most instances the maximal was

reached in a shorter period than before ergotoxine (cf. *b* and *d* with *g* and *h*, fig. 2), and the restoration period was not notably prolonged.

Response of some other organs than the heart to sympathetic action on smooth muscle. Since the effect of the agent brought to the heart by the blood stream, when we caused contraction of the pilomotor muscles, was similar to that produced by sympathetic impulses or adrenin (i.e., acceleration), the question naturally arose as to whether that agent would have corresponding effects on other organs innervated by sympathetic nerves.

A. Arterioles. As shown in figure 1, the blood pressure may be raised, in consequence of stimulating the pilomotor muscles, in such manner as to indicate that the rise is not due to shutting down a considerable vascular area by direct vasoconstriction from the stimulation, nor due to mere acceleration of the heart: the vascular area affected was not large; the rise of pressure did not develop promptly when the stimulation started and fall away quickly when it ended, but rose continuously and reached the maximal height only when a full minute had elapsed after the stimulus ceased, and then returned slowly to the original level; and though the blood pressure curve was shaped roughly like that representing the change in heart rate the point of highest pressure preceded the point of fastest rate. As previously mentioned, other records confirmed the relations demonstrated in figure 1. There were two experiments, however, in which the blood pressure was recorded, that showed little or no rise, although the tail hairs were made to stand erect and the heart rate was quickened by 12 or 14 beats. So many factors enter into the alterations of blood pressure that, under the circumstances, it cannot be regarded as an invariable indicator. For example, even an agent like adrenin, which is commonly regarded as a vasopressor agent, may have a purely vasodepressor action (see Cannon and Lyman, 1913). If the cardio-accelerator substance set free from smooth muscle acts similarly, it might cause a rise of pressure in some instances and no effect or even a fall in others. Although the facts illustrated in figure 1, therefore, demonstrate a striking elevation of blood pressure, which may reasonably be regarded as a consequence of

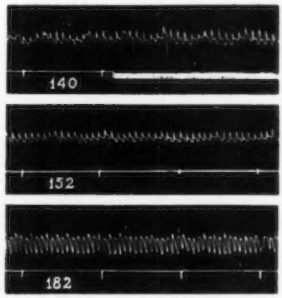

Fig. 3. Original records from the experiment of October 27, 1930. A. The start of stimulation of the nerves on the inferior mesenteric artery. B. Two and a half minutes after stimulating for 30 seconds, when a maximal increase of 12 beats was reached. C. The same stimulation as produced B, but applied 6 minutes after an intravenous injection of 1 mgm. of ergotamine (which had increased the rate to 150 beats per minute); the rate rose gradually and about one minute after stimulation reached 182.

the action of the substance arising from the pilomotor muscles, we would not wish to regard the phenomenon as being constant, like the response of the heart.

B. Denervated salivary gland. "The salivary glands of the cat are singularly susceptible to the action of adrenaline after denervation, and any stimulation which tends to promote outpouring of adrenaline into the blood, whether a reflex one, such as that caused by excitation of an afferent nerve, or a direct one, such as excitation of the splanchnics, causes a marked increase in the flow of saliva" (Sharpey-Schafer, 1924). Florovsky (1917), who has especially studied this peculiar phenomenon of the cat's submaxillary gland, found that the response was more prominent when it was superposed on the late stage of a flow produced by pilocarpine. Since this stimulation of the denervated salivary gland by adrenin was similar to the stimulation of the denervated heart by adrenin, we naturally decided to try the influence on the salivary output of exciting smooth muscle elsewhere in the body.

In performing these experiments we first abolished the sympathetic supply to the submaxillary gland on one side by removal of the superior cervical ganglion; also we removed one adrenal gland and denervated the other; after about ten days we cannulated the submaxillary duct and transected the spinal cord at the seventh thoracic vertebra in the morning, and in the afternoon, with the animal under curare, and when the effects of pilocarpine (1 cc. of 0.1 per cent, injected intravenously) were wearing off, we determined the influence of stimulating the caudal pilomotor nerves on the rate of secretion. In our first experiment we allowed the saliva to run along a horizontal glass burette, 40 cm. in length and 1 cc. in capacity, that was attached to the submaxillary duct. Since the burette was graduated in 0.01 cc. divisions the front of the flow could easily be noted as it advanced, and by use of a stop-watch the rate could be determined. We found that stimulation of the sympathetic nerves of the tail for one minute definitely and strikingly augmented the salivary secretion. Whereas the rate of flow before the stimulation was 0.01 cc. in 7 seconds, it rose in about two minutes to 0.01 cc. in 4.6 and 5.25 seconds—increases of approximately 50 and 33 per cent respectively.

In later experiments similar evidence was obtained by recording the drops. In figure 4 are reproduced the records of the intervals between drops. From the original continuous record excerpts were taken corresponding to the times indicated, and were turned to a vertical position so as to show graphically the changes wrought by stimulating the pilomotor muscles. It will be noted that the rate of flow was gradually diminishing when the stimulus (of 30 seconds) was applied, that by the end of the stimulation the flow was accelerating, and that the maximal rate—an increase of about 25 per cent—was reached about two minutes thereafter.

The result, in its time relations, is like that which is produced on the heart. We have obtained quite similar results on stimulating the nerves on the superior mesenteric artery after removal of the remaining adrenal gland, and also on stimulating the nerves arising from the inferior mesenteric ganglion. That the increased rate of salivary flow was not merely the consequence of a higher blood pressure and a larger volume flow of blood to the secreting gland, was proved by placing a loop around the aorta above the celiac axis, and closing that arterial trunk for 30 seconds. During the last 16 seconds one drop fell in 10 per cent less time than its predecessors, but the later drops fell at slower rates than the control.

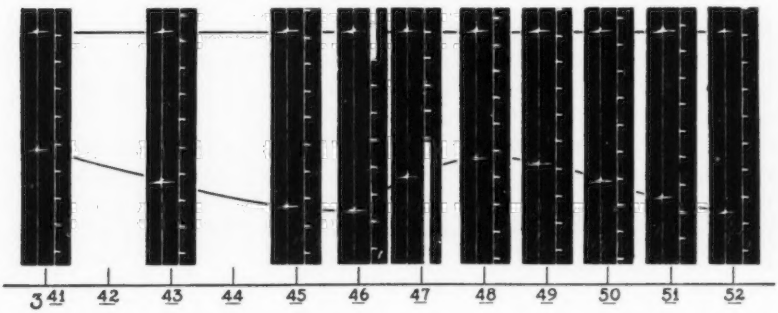

Fig. 4. Excerpts from a continuous record of drops of saliva, taken at the minutes indicated. In each instance the left line shows the drops of saliva, the right line the time (marked in two seconds). The rate of salivary flow was becoming slower (after pilocarpine), as shown by comparing 3:41 and 3:45. At 3:46–47 the sympathetic fibres to the pilomotor muscles of the tail were stimulated for 30 seconds. The maximal rate was reached about two minutes later—approximately 25 per cent faster than before the stimulation.

C. Denervated iris. We have had opportunity to make only two observations on the completely denervated iris, and unfortunately in both instances the pupil was so largely dilated that the results were not decisive. In one instance stimulation of the sympathetic supply to the caudal pilomotor muscles appeared clearly to cause the pupil to widen, but the method of measuring the small change was not sufficiently exact to be reliable. Further observations are needed. In this connection, however, it is interesting to note that Hartman, McCordock and Loder (1923), who have had much experience in using the wholly denervated iris as an indicator of medulliadrenal secretion, reported that in a cat excitement four hours after adrenalectomy caused dilatation of the denervated "pupil" within two minutes and that the pupil returned to its former size in about six minutes —time relations corresponding to those of the heart, blood pressure and

salivary glands when smooth muscle is stimulated. They also observed that in another case the pupil had twice its former width after stimulation of the pinna by induction shocks, although the adrenals had been extirpated. They attributed these results to increase of temperature (for which there was little evidence) or to the action of "some substance not produced by the adrenals." Elliott (1912) made observations which are similar, though in his experiments the iris was deprived of its sympathetic supply alone. He noted after excision of the adrenals that stimulation of the splanchnic nerves still caused slight dilatation of the pupil and slow withdrawal of the nictitating membrane on the sympathectomized side, "the reaction soon vanishing with repetition." Elliott adds, "It may be that the second effect, slight though it be, is similarly due to adrenalin, liberated from other paraganglia or from the actual processes of nervous excitation." These incidental observations by Hartman and his collaborators and by Elliott, we may reasonably infer, may belong with the phenomena which we have been describing.

D. Denervated stomach and intestines. We have performed only one experiment on the denervated stomach and intestines, but the conditions of that experiment were so excellent that it seems worth reporting. In a surgical preparation the ganglionic masses at the origin of the celiac axis and superior mesenteric artery were removed, right adrenalectomy was performed, and the left upper abdominal sympathetic chain was extirpated. A week later, on the day of the acute experiment, the animal was fed about 30 cc. of salmon and cream, and shortly thereafter was etherized long enough to permit transection of the spinal cord in the mid-thorax. Three hours later the sympathetic strands at the base of the tail were exposed; the abdomen was then opened by a mid-line incision, and the skin edges were elevated so as to make an open chamber with the stomach and the intestinal coils at the bottom. The viscera were kept moist by covering the elevated parts with wisps of cotton and pouring over them warm Ringer solution. Gastric peristalisis and rhythmic segmentation in the small intestine were proceeding vigorously. Stimulation of the sympathetic strands at the base of the tail caused a magnificent erection of the tail hairs, but there was no abolition of the activities of the gastro-intestinal tract. Repeated observation confirmed this result, and later, when records were made, they showed not even a lessening of the activities. It should be noted that the stomach and intestines are *inhibited* by sympathetic impulses.

A survey of the foregoing paragraphs reveals that the substance given off from smooth muscle has a stimulating effect not only on the heart, but on the arterioles, on the cat's salivary glands and probably on the iris. In all these instances the organs some time previously had been wholly deprived of their immediate sympathetic control, except the arterioles

which were denervated only in the fore limbs, the upper thorax and on one side in the upper abdomen, in the experiments on the heart. The gastro-intestinal tract, though denervated, was unaffected under circumstances which certainly would have stimulated a faster heart rate but, as just mentioned, the sympathetic *inhibits* instead of stimulating, gastro-intestinal activity.

Discussion. In the considerations now to be undertaken we propose to include not only the facts presented in this paper but also those of the preceding paper in which some of the same phenomena were described as a consequence of emotional excitement. Insofar as cardiac acceleration is concerned, the effects were quite the same in character whether smooth muscle was acted upon through its sympathetic fibres by emotional or by electrical stimulation, or whether the effect of electrical stimulation was contraction of smooth muscle (in pilomotor fibres) or inhibition (in the intestines). Comparison of the curves of the slow rise in figure 1 of the preceding paper (p. 378) with those in figure 2 in this paper (p. 398) shows a striking similarity, but just what might be expected. The chief difference is a larger effect under emotional stimulation, a fact which can be accounted for by the larger area of smooth muscle that was involved—the pilomotor muscles of much of the back as well as those of the tail.

The conclusion that the effects on the denervated heart, partly denervated blood vessels, and denervated salivary glands are due to a chemical substance given off by smooth muscle, is based on the following argument. We shall follow the argument with special reference to the heart, but most of the points are quite as pertinent to the other reacting structures. 1. The only connection of the heart with the rest of the body is by way of the blood; consequently the influence must be exerted by alteration of the blood. 2. As shown by numerous experiments described in the previous paper, the blood was not altered in a physical sense (i.e., by changes of temperature or pressure) to a degree which influenced the heart rate; nor was it altered chemically by the addition of metabolites from active skeletal muscles or the product of an endocrine gland—the cardiac acceleration continued after removal of all known sources of internal secretions or severance of their sympathetic connections. 3. When *a*, the sole remnant of the sympathetic supply to pilomotor muscles (i.e., those of the back) was abolished (see Newton, Zwemer and Cannon, 1931, p. 388), the acceleration disappeared; when *b*, the smooth muscle of the tail hairs, or the uterus and bladder, or the intestines (and the smooth muscle of the blood vessels in each instance), was acted on by sympathetic impulses the typical result followed; and when *c*, the return of blood from the tail was blocked during stimulation of its pilomotor muscles the acceleration was greatly reduced, or delayed, until the block was lifted. 4. The effect is not due to liberation of adrenin from sympathetic ganglia, where a small

number of chromaffine cells may be found, or from paraganglia in the lower aorta, for the typical cardiac response appears when the postganglionic sympathetic strands at the base of the tail or postganglionic fibres on the inferior mesenteric artery are stimulated. These results also rule out possible involvement of coccygeal gland. Emotional excitement or electrical excitation, therefore, that discharges sympathetic impulses into smooth muscle, causes a substance to be produced which, escaping into and carried by the blood stream, is capable of affecting distant viscera.

We have ventured to call the substance produced by smooth muscle a hormone because it fulfills the qualifications laid down by Starling (1914) in suggesting that term,—it is formed in certain cells under normal conditions, it is carried by the blood to remote organs, and it acts there in a way favorable to the organism, for it arises when the sympathetic is active and affects these organs as the sympathetic affects them. In other words, it acts in the same direction as sympathetic impulses, and in their absence is capable of bringing denervated organs into conformity with those which are innervated, so that the characteristic unified response occurs.

The thoughtful reader has probably recognized already that the action of the substance given off from smooth muscle, when influenced by sympathetic impulses, resembles that of adrenin. A substance which acts similarly was described by Loewi (1921, 1922) as being produced by cardiac muscle of frogs and turtles when stimulated through its accelerator fibres; Ringer solution in contact with the heart muscle during sympathetic stimulation caused, when applied to a second heart, a characteristic increase of its contractions. The accelerator substance is destroyed when the cardiac contents are ashed, and, like adrenin, it is rendered inactive when mixed with eosin and exposed to ultraviolet light (Loewi and Navratil, 1926). Although a considerable number of investigators have failed to confirm the results described by Loewi (see review by H. Fredericq, 1927), the positive testimony has become overwhelming. Furthermore the charged Ringer solution can affect not only the heart but also smooth muscle as adrenin affects it; Brinkman and Van Dam (1922) have reported that the solution taken from the excited heart of frog A causes inhibition of the stomach movements of frog B, an observation confirmed by Lanz (1928*a*) and by Külz (1928). Again, like adrenin, the charged solution causes constriction of the frog's arterioles; like adrenin it is rendered more effective by a dilute solution of glycocol; and like adrenin it loses its activity on standing in air for 24 hours or on being heated to 100° C. (Lanz, 1928b). This interesting substance is not derived solely from the heart of cold-blooded animals, for Loewi's experiments have been repeated on a mammal; by perfusing the cat heart with defibrinated blood while stimulating its sympathetic nerve supply Rylant (1927) succeeded in altering

the blood so that it caused in a second isolated cat heart an acceleration of the beats and an augmentation of their amplititude. And finally, Finkelman (1930) has recently reported that Ringer solution running over a pulsating piece of rabbit intestine, A, still supplied with its mesenteric nerves, and passing to another pulsating piece of intestine, B, acquires a new property when the nerves are stimulated—the piece A is inhibited and thereupon the piece B is inhibited also. A second stimulus is quite as effective as the first on piece A, but not on piece B (cf. p. 393 supra). Ephedrin antagonized both inhibitions and also antagonized inhibition produced by adrenalin—i.e., there was that indication that the secondary effect was due to an adrenin-like substance carried by the Ringer solution from piece A to piece B. Finkelman speaks of the substance as an "inhibitory substance" but obviously that effect, like the effect of adrenin, depends on the region of action; at the intestine it inhibits, but, as our experiments show, at the heart it stimulates.

Although there is this cumulative evidence of a chemical agency, resembling adrenin in its action, that is given off from cardiac muscle (and from rabbit intestine) when acted upon by sympathetic impulses, there has been no clear proof that it is a phenomenon that occurs in natural conditions. As Demoor (1929), although arguing for the reality of humoral transmission of nerve impulses, has remarked: "il est évident que les irrigations du coeur qui interviennent dans toutes ces recherches, peuvent créer des conditions de vie tissulaire nouvelles, accompagnées de perméabilités qui n'existent pas physiologiquement, et que les émigrations des substances vagale et sympathique, expérimentalement constatées, peuvent ne pas intervenir à l'état normal." The results described in the previous paper and in this one have furnished evidence—the first evidence, so far as we have been able to learn—that cells other than those of endocrine glands, that are innervated by sympathetic neurones, give off into the circulating blood, in the course of quite natural behavior, a peculiar substance capable of influencing in a characteristic manner other cells having a sympathetic nerve supply.

Nothing we have observed is opposed to the view that the substance produced by action of sympathetic impulses on smooth muscle is adrenin or an adrenin-like substance. Indeed, the remarkable and peculiar ability of adrenin to cause a free flow of saliva from the cat's submaxillary gland and the similar ability of the substance given off by smooth muscles, when considered in relation to its cardio-accelerator action, points quite directly towards its being adrenin. If it is adrenin, however, it has a different immediate source than that discharged from the adrenals and should be distinguished, therefore, from adrenin. Lanz has designated it as the "substance sympathique." We would suggest that it be called provisionally *sympathin*, with the understanding that as knowledge of its character

increases it may prove really to be adrenin, developed for local action in smooth muscle cells.

The view that sympathin may be the same substance chemically as adrenin is supported by the evidence that as time passes after removal of sympathetic ganglia connected with an organ the organ becomes more sensitive to both substances. The reader will recall that as time passed after denervation of the heart the rises of pulse rate became gradually higher during the first few days (see p. 379, previous paper), and that in acute experiments we rarely obtained more than a single slight response from the denervated heart (see pp. 393, and 399, supra), whereas the heart denervated a week or ten days before the test responded vigorously many times (see fig. 2). In both conditions the *smooth muscles* were freshly prepared—the difference lay in the different periods of denervation of the heart before it was used as a test organ. The effect of destruction of the immediate sympathetic supply to an organ in causing it after a few days to become more sensitive to adrenin was recognized by Elliott (1905), and has been proved for the denervated iris, denervated blood vessels of the rabbit ear, denervated retractor penis of the dog, and denervated arrectores pilorum. It is quite possible that the rising sensitiveness of an organ after being denervated results from accumulation of sympathin within the idle cells—when touched off, there is more sympathin present to act in coöperation with adrenin. The organs, in addition to the heart, that we have used for tests were all denervated. It is peculiarly interesting, in view of the failure of full contraction of the muscles of the tail hairs to stop gastro-intestinal movements (see p. 404) that Elliott did not observe any "greater ease of inhibition as the sequel to denervation."

The evidence that a substance having an adrenin-like influence is given off from smooth muscles when they are acted on by sympathetic impulses recalls an interesting idea expressed by Elliott (1904). Struck by the fact that adrenin has its usual influence after the sympathetic fibres to smooth muscle have degenerated, he concluded that adrenin does not act by exciting any structure derived from or dependent for its existence on the peripheral neurone, and he suggested that the muscle cell, in response to its subjection to sympathetic control, develops a local mechanism the function of which is to receive and transform the nerve impulses, and that adrenin might be the chemical stimulant liberated whenever the impulse arrives at the periphery. According to this hypothesis adrenin, discharged into the circulating blood from the adrenal medulla, would mimic the action of sympathetic impulses because it is the same substance as that which mediates the action of nerve impulses on the special mechanism (contraction or inhibition) in the effector cell. By a small extension of this hypothesis the phenomenon of humoral transmission of sympathetic impulses from smooth muscle to other structures under sym-

pathetic control could be explained; it might be the consequence of so much local adrenin liberated by the impulses that it overflows the cell boundaries and enters the blood stream. And finally, on this hypothesis, even with a delicate responsive organ erection of the hairs might occur without distant humoral effects, for the local adrenin, though sufficient to set going the local contractile mechanism, might be insufficient to overflow. When it passes out of the cell it acts more slowly than the adrenin from the adrenal medulla, but that may be explained 1, by a possibly slower permeability of the wall of the muscle cell as compared with that of the secreting medulliadrenal cell; 2, by the distance through which the sympathin must diffuse in tissue fluid before reaching a capillary wall; 3, by the necessity of traversing that wall in order to enter the circulation (whereas the medulliadrenal cells abut on the vascular sinuses and probably secrete adrenin directly into the blood (Sharpey-Schafer, 1924)), and 4, by the vasoconstriction of peripheral areas from sympathetic stimulation as compared with the vasodilatation of the adrenals when the splanchnics are excited (Biedl, 1897).

In relation to Elliott's surmise, the action of ergotamine in our experiments is of interest. This drug stops the positive action not only of sympathetic impulses but also of adrenin. As already mentioned, however, it is much less effective in this respect on heart muscle than on smooth muscle, e.g., of the blood vessels. An amount of ergotamine which prevents sympathetic impulses from causing contraction of pilomotor muscles does not prevent these muscles from discharging sympathin into the blood stream and thus accelerating the heart (see fig. 2, *j* and *k*). It would appear, therefore, that ergotamine interposes a block between sympathin and the contractile apparatus of the pilomotor cell (i.e., that sympathin is derived from the excitatory process, not from the contractile process), and that the sympathin which does not act locally can escape and act at a distance, i.e., on the heart, because the contractile apparatus of the heart still remains sensitive to it.

Elliott (1905) reported observations which led to the conclusion that adrenin "disappears in the tissues which it excites." The attractive idea is suggested that adrenin may be produced originally in the adrenal medulla and that the local stores in smooth muscle are derived from the central station. That view we have not been able to support. In one instance when the response of the heart to stimulation of the tail hairs was small (increases of only 9 and 6 beats per minute) we injected 0.25 cc. of adrenalin (1:100,000) and the cardiac rate increased 64 beats per minute. Then we injected rapidly 1 cc. of the solution. When the effect passed off we again injected, at the same rate as before, 0.25 cc.; the pulse rose 68 beats per minute. Evidently the large injection had not lessened the sensitiveness of the heart. Now we stimulated again, precisely as before, and though the

tail became "bushy," the heart rate increased only 2 beats, i.e., if the adrenalin disappeared in part into the muscles of the tail hairs, it did not result in a larger output into the blood stream when these muscles were stimulated. This negative result is confirmed by observations on cats with one adrenal removed and the medulla of the other extirpated. Many months after that operation emotional excitement causes the characteristic slow increase of the rate of the denervated heart. We infer, therefore, that, if the substance which we have called sympathin is adrenin, it is produced locally in the cells under sympathetic control and is not derived from the adrenal glands.

Since the evidence in hand indicates that sympathin and adrenin are liberated simultaneously by the same agency (sympathetic impulses), and have similar results on organs under sympathetic government, it is clear that when, in previous reports of sympathetic activity, effects have been ascribed to adrenin alone, sympathin must be understood as being present and coöperating. And when, after exclusion of sympathetic and medulli-adrenal influences on an organ, typical sympathetic responses nevertheless have occurred, the possibility that sympathin was acting should be recognized. Thus Griffith (1923) noted, after cutting the nerves to the liver and suppressing adrenal participation, that excitement still caused a rise of blood sugar. And Houssay, Lewis and Molinelli (1928) found that morphine caused a hyperglycemia after cutting the liver nerves and excluding the influence of the adrenal medulla. In both series of experiments sympathin may have been the effective factor.

Finally we wish to call attention to the support which the observations in these papers offer to the view that the sympathetic system is arranged to work as a unified and integrated whole (see Cannon, 1915, 1930). The distribution of preganglionic fibres to numerous outlying neurones in a series of sympathetic ganglia, and the diffuse distribution of adrenin in the circulating blood, point definitely to that mode of functioning. The evidence now presented shows that, as a rule and possibly always, excepting cases of inhibition, even if there should be only local activity of the sympathetic, such activity may call inactive parts into operation, and that in case of paralysis of a part from lack of nerve impulses, the rest of the system may nevertheless force coöperation.

SUMMARY

In a cat with heart denervated, adrenals and liver excluded from action and spinal cord severed in the mid-thoracic region (so as to permit, without anesthesia, painless operations in the hind part of the animal), stimulation of the sympathetic supply to the smooth muscle of the tail hairs (and of the blood vessels of the region) causes, for about two minutes, a gradual increase of blood pressure (see fig. 1), a gradual increase of heart rate (see

figs. 1 and 2) and a gradual increase of salivary secretion (see fig. 4). The time relations of the increase and the slow return to the former state are, in each instance, similar to those reported for the denervated heart alone, in the previous paper, as a consequence of emotional excitement.

If the blood flow into and from the legs and tail is hindered, the phenomenon is much reduced (see fig. 2, *e*) or is delayed until the flow is restored (see fig. 1). Since the only tissue known to be affected by sympathetic stimulation at the base of the tail or on the mesenteric arteries is smooth muscle, since the only connection between the hind part of the animal and the responding denervated organs in the fore part is the blood stream, and since interfering with the stream in the region where smooth muscle is stimulated either markedly depresses or abolishes the response, the inferences are drawn that a substance is given off from smooth muscle into the blood, that it is carried effectively by the blood to distant organs, and that it influences those organs in a favorable manner, i.e., as sympathetic impulses would influence them.

Because the properties just mentioned are those of a hormone we regard the substance given off by smooth muscle, when affected by sympathetic impulses, as a hormone.

The substance arises not only from pilomotor muscles, but also from the smooth muscle of the intestine (see fig. 3), and the bladder and uterus.

Because the substance is derived from structures under sympathetic control, when they are influenced by sympathetic impulses, we suggest that it be called *sympathin.*

Sympathin is given off from the smooth muscle of the tail and can increase the heart rate after ergotamine has prevented sympathetic impulses from causing erection of the tail hairs (see figs. 2, *j* and *k*).

The bearing of these observations on those of Loewi and others on the humoral transmission of nerve impulses, the support which the observations bring to some early views of Elliott, and the possible relations of sympathin and adrenin, are considered in the discussion.

BIBLIOGRAPHY

Biedl, A. 1897. Pflüger's Arch., lxvii, 443.

Brinkman, R. and E. Van Dam. 1922. Ibid., excv, 66.

Cannon, W. B. 1915. Bodily changes in pain, hunger, fear and rage. New York.

1930. The Linacre Lecture. The Lancet, London, May 24, 1100.

Cannon, W. B. and H. Lyman. 1913. This Journal, xxxi, 376.

Cannon, W. B., J. T. Lewis and S. W. Britton. 1926. Ibid., lxxvii, 326.

Dale, H. H. 1906. Journ. Physiol., xxxiv, 176.

Demoor, J. 1929. Ann. de Physiol. et de Phys.-Chimie, v, 60.

Elliott, T. R. 1904. Journ. Physiol., xxxi, p. xx.

1905. Ibid., xxxii, 401.

1912. Ibid., xliv, 374.

Finkelman, B. 1930. Ibid., lxx, 145.

FLOROVSKY, G. B. 1917. Bull. de l'Acad. Impér. des Sci., Petrograd, ix, 119.
FREDERICQ, H. 1927. C. r. Soc. de Biol., Paris, xcvi, S. P. 3.
GRIFFITH, F. R. 1923. This Journal, lxvi, 618.
HARTMAN, F. A., H. A. MCCORDOCK AND M. M. LODER. 1923. Ibid., lxiv, 1.
HOUSSAY, B. A., J. T. LEWIS AND E. A. MOLINELLI. 1928. C. r. Soc. de Biol., xcix, 1411.
KÜLZ, F. 1928. Arch. f. exp. Path. u. Pharm., cxxxiv, 252.
LANGLEY, J. N. 1894. Journ. Physiol., xv, 176.
LANZ, A. B. 1928a. Arch Néerland. de Physiol., xii, 433.
1928b. Ibid., xiii, 423.
LOEWI, O. 1921. Pflüger's Arch., clxxxix, 238.
1922. Ibid., cxciii, 201.
LOEWI, O. AND E. NAVRATIL. 1926. Ibid., ccxiv, 678.
MELTZER, S. J. AND J. AUER. 1908. This Journal, xxi, 400.
NEWTON, H. F., R. L. ZWEMER AND W. B. CANNON. 1931. Ibid., xcvi, 377.
RYLANT, P. 1927. C. r. Soc. de Biol., Paris, cxvi, 1054.
SHARPEY-SCHAFER, E. 1924. The endocrine organs. London, Pt. 1, 138.
STARLING, E. H. 1914. Proc. Roy. Soc. Med., vii, 29.

A PROBLEM IN THE COAGULATION OF THE BLOOD

"Sweet Clover Disease of Cattle"

LEE M. RODERICK

From the North Dakota Agricultural Experiment Station

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The attention of research workers on animal diseases was directed some years ago to the occurrence of losses among cattle which were fed on hay or silage made from sweet clover. Such forage has been found to be of poor quality for it was spoiled, moldy and damaged. It is evident that the sweet clover acquires specific disease-producing properties when it spoils in the curing process. Sweet clover disease which is the clinical entity which results from the feeding of such spoiled feed has been found only on those farms where that forage was used for feed and it is regularly produced by the feeding of cattle and rabbits with the hay or silage secured from farms where the losses have occurred. Properly cured sweet clover appears to be quite free from danger inasmuch as it may be fed to animals for long periods without the appearance of the specific alteration in the blood. Serious losses are reported from time to time with the condition so that the problem has an economic significance.

The disease is characterized by the development of a gradually increasing prolongation in the time required for the blood of the animals to coagulate when it is drawn into tubes or in the bleeding time of injuries and wounds. When the condition proceeds to the usual fatal termination, it is from hemorrhage either in the tissues of the body or from surgical or accidental wounds, as shown by these illustrations. A cattleman dehorned 80 calves fed on sweet clover and 65 died of hemorrhage within a day or two. The active, well developed calf born of a cow fed for 13 days on damaged sweet clover died of hemorrhage 28 hours after birth. Twenty-five young bulls, varying in age from 8 to 14 months were castrated and 12 died of internal hemorrhages.

The work reported here deals primarily with the study of the alterations involved in the coagulation of the blood. Obviously, the clinical, microbiologic, agronomic and pathologic aspects of the problem cannot be presented here. Further information on those aspects is included in another publication (Roderick and Schalk, 1931). Certain data are interesting from a physiologic viewpoint so that this phase of the work is presented in order that it may come directly to the attention of physiologists.

DELAYED COAGULABILITY OF THE BLOOD. *Determination of the clotting time.* Probably the first recognizable indication of the deleterious effect of the sweet clover is the appearance of the prolongation in the coagulation time of the blood. The time as determined by the routine method followed usually requires from 12 to 20 minutes for the blood of normal cattle to clot. The condition presents itself when such clotting times are increasingly prolonged so that by the time of the advent of hemorrhage the blood is usually of itself completely incoagulable when drawn into test tubes and held in the water bath at 37°C. Clotting is not considered to have occurred until the blood remains in the inverted tube. There is usually little evidence of change in the blood of cattle until about ten days after the start of feeding and in a series of animals with various hays, the hemorrhage has not appeared sooner than three to five weeks. Rabbits ordinarily die in 6 to 18 days. If the feeding of a sufficiently damaged hay is continued the condition invariably proceeds to hemorrhage in which the mortality in untreated animals is nearly 100 per cent.

The analysis of a condition involving delayed coagulation of the blood may assume the possibility of 1, the decrease of some constituent necessary for the coagulation, or 2, the presence or increase in some factor which acts in an inhibitory manner, or 3, an alteration in the physical inter-relationships in the blood. This study deals with the possibility of a 1, diminished fibrinogen content of the blood; 2, a deficiency in prothrombin; 3, an excess of antithrombin; 4, a deficiency of calcium ions; 5, a diminution in the platelets or a deficiency in their lipoidal fraction.

Fibrinogen. The early work on this problem suggested that the blood of these sweet clover calves contained a sufficient amount of fibrinogen to insure the formation of a clot which readily contracts if an adequate quantity of thrombin is added. The addition of a few drops of tissue extract or of freshly defibrinated blood, even of the animal itself which may be secured by prolonged shaking, is effective in promptly and firmly clotting 5 cc. of the incoagulable blood. The method of Gram (1921) was used in the determination of the fibrin yields from calcified citrated plasma. It soon became necessary to add thrombin to supplement the simple calcification of the plasma and thereby secure the complete coagulation of the fibrinogen. One or two cubic centimeters of bovine serum with a little cephalin solution was added to 5 cc. of the plasma diluted for that purpose.

The accompanying graph indicates that there was some diminution in the plasma fibrinogen in the course of the feeding experiments. The lowest amounts found, however, were within the range of values secured with normal animals. A series of determinations was made on 24 normal cattle of varying ages which averaged 0.65 per cent of fibrin per 100 cc. of blood plasma. They varied from 0.43 to 0.92 per cent.

The work of Foster and Whipple (1921–22) indicates that the only con-

dition which without exception is associated with the low fibrin yields is acute extensive liver injury such as results from chloroform and phosphorus poisoning. The further possibility of serious liver injury is considered in another paper (Roderick, 1929). One hesitates to attribute the slight decrease in fibrinogen in the sweet clover calves to serious liver in-

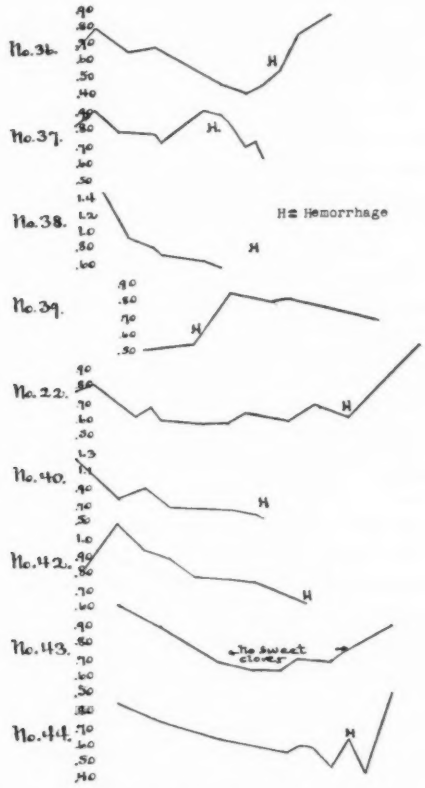

Fig. 1. Percentage of fibrin yields from bovine plasma during sweet clover feeding experiments.

jury nor does this comparison suggest that a deficiency in fibrinogen is concerned in the delayed coagulation. It rather indicates that the fibrinogen level varies and depends on a number of factors. Foster and Whipple (1921–22) observed simple hemorrhage to cause a distinct over-production of fibrinogen. Recovery from hemorrhage in these experimental animals

is followed by an increase in the blood fibrinogen over the quantity in the animal when normal.

Prothrombin. This phase of the work was primarily one of the quantitative comparison of the prothrombin in the blood of bovines fed on damaged sweet clover with that of normal animals.

The suitable recalcification of oxalated normal plasma produces prompt coagulation. Howell (1914) suggested that this procedure serves to bring into active form the maximum supply of prothrombin present in the blood. It was found, however, that the coagulation of recalcified oxalated plasma of sweet clover calves required a gradually prolonged period and finally failed completely to coagulate on the simple addition of calcium (blood 10 cc. plus 20 mgm. sodium oxalate). Trials were made with 5 drops or 0.4 cc. of the plasma with respectively 1, 2, 3, 4 and 5 drops of 0.5 per cent $CaCl_2$ solution in small tubes in the water bath at 37°C. The following table shows the progressive change in the blood as reflected by the recalcified plasma as compared with the clotting time of the blood.

Calf 77. Started feeding sweet clover May 8, 1928.

5/8. Oxalated plasma clotted in 4 mins. Blood clotted in 8 mins.
5/11. Oxalated plasma clotted in 6 mins. Blood clotted in 12 mins.
5/15. Oxalated plasma clotted in 7 mins. Blood clotted in 45 mins.
5/19. None clotted. Blood clotted in 46 mins.
Failed to clot on later dates. Blood clotted 5/26 in 67 mins. Blood clotted 5/29 in 106 mins.

Internal hemorrhage June 1, 1928, with incoagulable blood.

Many observations on a series of calves suggest that the delay in the coagulation of recalcified oxalated plasma develops constantly and parallels quite closely the retardation in the clotting time of the blood. A prolongation in the prothrombin time, therefore, regularly appears but it is not related to the amount of calcium originally present in the blood nor does the addition of cephalin induce the clotting of such plasma. In like manner, it was found that the recalcification of citrated plasma becomes delayed as the feeding of the damaged sweet clover proceeds and eventually it too fails to clot.

Solutions of prothrombin, prepared by precipitation of normal bovine plasma with the acetone method of Howell (Howell and Cekada, 1926), when added to the sweet clover blood, promoted coagulation. On the other hand, similar preparations of prothrombin made at the same time in parallel manner from sweet clover blood were quite ineffective in producing coagulation.

Prothrombin so prepared from the blood of a sweet clover calf was added to its own incoagulable blood.

Tubes	1	2	3	4
Prothrombin calf 44	0.5 cc.	1.0 cc.	2.5 cc.	0
Blood of calf 44	5.0 cc. to each tube			
Temp. 37°C. No coagulation in 6 hours.				

The addition of a parallel preparation of prothrombin, which was made from normal bovine blood to 5 cc. of the blood of the same sweet clover calf did, however, produce coagulation.

Tubes	12	13	14
Prothrombin normal calf 4	0.5 cc.	1.0 cc.	2.5 cc.
Blood of sweet clover calf 44	5 cc. to each tube		
Clotted	1 hr.	45 min.	20 min.

The addition of 0.25 cc. of cephalin solution in a similar test reduced the coagulation times to 33 minutes, 10 minutes, and 8 minutes respectively.

It was found, moreover, that when a prothrombin solution from normal blood is added to recalcified sweet clover plasma which in itself fails to clot on recalcification, it will clot like normal plasma.

Plasma-sweet clover calf 44; 5 drops + 3 drops 0.5 per cent $CaCl_2$ solin + 2 drops normal prothrombin solution; clotted in 7 minutes.

On the other hand, if the prothrombin solution is made from the blood of a sweet clover calf, it was found to be relatively ineffective. The addition of cephalin did not promote coagulation. In the following experiment, the prothrombin had been previously made from the animal's own blood. In such comparative work, the prothrombin solutions were prepared from the dried stable acetone prothrombin precipitates.

PLASMA CALF 44	CALCIUM CHLORIDE SOLUTION	CEPHALIN SOLUTION	PROTHROMBIN CALF 44	
5 drops	3 drops	2 drops	1 drop	None clotted in 24 hours
5 drops	3 drops	2 drops	2 drops	
5 drops	3 drops	2 drops	5 drops	
5 drops	3 drops	2 drops	10 drops	

Comparative trials with solutions of fibrinogen likewise show less effectiveness for coagulation with the preparations of prothrombin made with sweet clover blood than for those from normal blood. Such prothrombin solutions prepared by acetone precipitation do not contain antithrombin, according to Cekada (1926).

Prothrombin prepared from normal blood

	TUBE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Drops fibrinogen	8	8	8	8	8	8	8	8	8	0
Drops prothrombin no. 4	2	3	4	5	6	7	8	0	8	8
0.5 per cent $CaCl_2$ solution	3	3	3	3	3	3	3	3	0	3
Clotted, mins	46	35	27	27	23	20	20	—	—	—

The above experiment repeated with the addition of one drop of cephalin to each tube reduced the clotting time of all tubes to 8 minutes. The following experiment performed the same day is representive of the ineffectiveness of those prothrombin solutions prepared from sweet clover blood. The same fibrinogen was used in both tests.

	TUBE NUMBER									
	1	2	3	4	5	6	7	8	9	10
Drops fibrinogen	8	8	8	8	8	8	8	8	8	0
0.5 per cent $CaCl_2$ solution	3	3	3	3	3	3	3	3	0	3
Drops prothrombin no. 39	2	3	4	5	6	7	8	0	8	8

None clotted in six hours. A similar test was made with the addition of 1 drop of cephalin to each tube. Clotting occurred in 151 minutes for the smallest amount of prothrombin and in 56 minutes with tube 7. Similar tests made with prothrombin preparations made from the same animal at the start of the feeding trial, later with the slowly coagulable blood and finally with the incoagulable blood likewise showed the progressive diminution in prothrombin.

Further evidence that a deficiency of prothrombin is involved was shown when a bovine with incoagulable blood was given an intravenous injection of prothrombin prepared with Howell's acetone precipitation method. Precipitation was made of normal plasma and a solution made from the dried preparation about five months later. The quantity given represented about 450 cc. of the original plasma. It promptly reduced the clotting time to a period average of 30 minutes.

This work shows that the delayed coagulability of sweet clover blood involves a reduction in the prothrombin of the blood. The diminution parallels the delay in the coagulation time. The origin of the plasma proteins with the exception of the fibrinogen is still obscure so that no attempt will be made in this paper to explain the pathologic process whereby the prothrombin, which is a pseudoglobulin (Cekada, 1926), is diminished by the feeding of damaged sweet clover.

Antithrombin. The following experiments were for the purpose of detecting the possible presence of increased amounts of principles which

might delay the coagulation of the sweet clover bloods. Such blood when added to normal blood does not exert any appreciable inhibitory action on the coagulation. On the other hand, a small quantity of normal blood appears to supply the deficiency of sweet clover blood so that coagulation proceeds at the normal rate.

The blood of a rabbit which had been fed on highly "toxic" sweet clover for 9 days, was drawn from the heart with an oiled syringe and added to normal rabbit blood similarly obtained. Hemorrhage was subsequently found on the wall of the thorax and stomach on necropsy of the rabbit.

	TUBE NUMBER							
	1	2	3	4	5	6	7	8
Normal rabbit blood, cc.	2.0	2.0	2.0	1.0	0.1	2.0	0	0
Sweet clover blood, cc.	0.1	1.0	2.0	2.0	2.0	0.	2.0	2.0
Clotting time, mins.	7	7	5	6	9	9	40	45+

Temp. 37°C.

An attempt was made to prepare heparin or to isolate an inhibitory principle from the slowly coagulating blood of sweet clover calves. Quantities of 200 cc. of blood from each of three such calves was precipitated with acetone according to the method of Howell (1926). The final residue was extracted with 4 cc. of hot saline and the blood of a normal calf was used to check the inhibitory effect of the extracts. Trials with several preparations failed to show the presence of any increased amount of a substance which would delay coagulation. The action of the preparations did not differ from those prepared with normal blood. The writer concluded that bovine blood contains little heparin when compared with dog blood.

The blood of sweet clover calves was further tested for the presence of antithrombin by the following method. The oxalated plasma was slowly heated to 54–55°, centrifugalized and tested as shown but no apparent increase in antithrombin was demonstrated for the sweet clover blood.

	NORMAL CALF 4				SWEET CLOVER							
					Calf 39				Calf 41			
Drops of fibrinogen	8	8	8	8	8	8	8	8	8	8	8	8
0.5 per cent $CaCl_2$ solution	3	3	3	3	3	3	3	3	3	3	3	3
Drops preparation antithrombin	1	2	5	10	1	2	5	10	1	2	5	10
Incubation for 10 minutes												
Drops of prothrombin	7	7	7	7	7	7	7	7	7	7	7	7
Clotting time, mins.	15	15	30	120	15	15	30	130	15	15	30	130

Moreover such antithrombin preparations from sweet clover blood do not delay the coagulation of normal oxalated plasma when recalcified appreciably more than similar preparations from normal blood.

PLASMA NO. 4	$CaCl_2$ SOLUTION 0.5 PER CENT	ANTITHROMBIN	
Antithrombin no. 4 (normal)			
5 drops	2 drops	2 drops	All clotted in 11 minutes
5 drops	3 drops	3 drops	
5 drops	4 drops	4 drops	
5 drops	5 drops	5 drops	
Antithrombin no. 42 (sweet clover)			
5 drops	2 drops	2 drops	All clotted in 11 minutes
5 drops	3 drops	3 drops	
5 drops	4 drops	4 drops	
5 drops	5 drops	5 drops	

Nothing has therefore been found to indicate that the delayed coagulation is due to the increase of an inhibitory principle.

The blood calcium. It is experimentally proved that calcium is necessary for the proper functioning of the clotting mechanism. Deficiencies of calcium however do not seem to have been reported with abnormalities in coagulation in other conditions.

Work on nutrition and mineral metabolism indicates that the level of blood calcium is quite constant in bovines whether the calcium balance is negative or positive (Hart et al., 1920). Available data on bovine blood calcium involves animals of different ages, conditions of pregnancy, lactation and diet. Robinson and Huffman (1926) reported 117 determinations ranging from 7.7 to 14.7 mgm., average 11.0 mgm. of calcium per 100 cc. of plasma. Meigs, Blatherwick and Cary (1919) found an average of 9.72 mgm. while Hart et al. (1922) found one of 16.25 mgm. of calcium per 100 cc. of serum.

Quantitative estimations. The blood calcium of three experimental calves was determined at intervals by the method of Clark (1921). The work was started about the time the first delay was observed in the coagulation of the blood. Six subsequent determinations were made on the slowly clotting blood of those cattle. The blood calcium varied from 11.1 to 18.5 mgm. average 13.1 mgm. per 100 cc. of plasma. Eight determinations were made on the slowly clotting blood of five additional calves which gave an average of 6.96 mgm. with a range of 5.79 to 9.02 mgm. per 100 cc. of blood.

The blood calcium of calves of which the blood failed to clot for hours showed little variation from that at the beginning of the experiment or

that of normal animals. The above figures likewise conform to the range given by others. This indicates that the blood, which remains unclotted for hours, contains normal amounts of calcium. This does not indicate that the delay in the coagulation is the result of oxalate poisoning in the cattle. The functional pathologic studies (Roderick, 1929) further substantiate that conclusion.

The addition of calcium to such blood does not accelerate the process of coagulation.

Blood of calf 8804

15 cc. of blood + 0.1 cc. of 2% $CaCl_2$ sol'n, clotted in 76 min.
15 cc. of blood + 0.2 cc. of 2% $CaCl_2$ sol'n, clotted in 79 min.
15 cc. of blood + 0.5 cc. of 2% $CaCl_2$ sol'n, clotted in 79 min.
15 cc. of blood + 0.5 cc. of 2% $CaCl_2$ sol'n, plus 0.2 cc. cephalin 84 min.
15 cc. of blood control, clotted in 76 min.

The intravenous injection of a solution of 7.5 grams of $CaCl_2$ and 10 grams of dextrose in 750 cc. of salt solution did not improve the clotting time of the incoagulable blood of an experimental bovine. Nothing has been found, therefore, to suggest that the blood calcium is not available in sufficient quantity for the coagulation of the sweet clover blood.

The blood platelets. It was assumed in the beginning of this study that a deficiency of platelets might explain the characteristic delay in the coagulation. Schofield (1924) noted a reduction in the number of platelets in some of his observations. The possibility of this alteration is logical on the basis of analogy for a reduction occurs in aplastic anemia (Larrabee, 1920) and (Duke, 1928). The diminution in platelets is associated with a hemorrhagic diathesis in purpura hemorrhagica (Rosenthal, 1928). Hurwitz and Drinker (1915) found that benzol poisoning reduced both the platelets and the prothrombin but they could not establish a parallel between the extent of bone marrow injury, the number of blood plates and the relative amounts of prothrombin.

Writers differ in their opinion relative to the rôle of the platelets in the mechanism of coagulation. Bayne-Jones (1912) believed them to be the source of prothrombin. Bordet (1921) and Mills (1927) on the other hand believe the prothrombin principle to be a property of the plasma alone.

The platelets are no doubt a source of the lipoidal principle which functions in the process of coagulation. Cephalin when added to the sweet clover blood does not accelerate the delayed coagulation. Previous mention has been made of the failure of cephalin alone to aid the clotting of recalcified sweet clover plasma. It was found to accelerate the clotting action of added prothrombin but it by no means aided a deficient preparation to function as promptly in experimental coagulation as a potent preparation from normal blood.

Platelets were obtained from oxalated and citrated normal bovine plasma and washed with the corresponding anticoagulant. They were emulsified in salt solution and in distilled water but they failed to clot sweet clover plasma except by the addition of normal prothrombin.

Tubes of 0.4 cc. of oxalated plasma + 3 drops of 0.5 per cent $CaCl_2$ solution + 1 to 10 drops of an aqueous suspension of platelets failed to clot in one hour.

Tubes with 0.4 cc. of oxalated plasma + 3 drops of normal prothrombin + 3 drops of 0.5 per cent $CaCl_2$ solution clotted in 12 minutes. This with the addition of one drop of platelet suspension clotted in 5 minutes.

A study of the influence of damaged sweet clover on the number of platelets during the feeding experiment was made by a modified Wright and Kinnicut technic. Quite satisfactory results were secured if about 0.1 cc. of 40 per cent formaldehyde solution was added to 50 cc. of fresh (1–1400) potassium cyanide solution. Three parts of that solution were then mixed with two parts of (vital) brilliant cresyl blue solution of a dilution of 1–300. Little precipitation occurred in the hemacytometer if about two minutes elapsed before filtration and if the solution was used within a reasonably short time. The data follow below with the clotting time of the blood for comparison.

No. 8830
Started feeding 2–19–'24

4–14—182000..... 56 min.
4–18—214000..... 55 min.
4–21—360000..... 31 min.
5–7 —616000..... 70 min.
5–8 —590000.....
5–12—456000..... 60 min.
5–27—568000.....
5–31—398000..... 3 hrs.+
6–2 —102000..... 2 hrs.+
Dead of hemorrhage 6/2/24.

No. 9750
Started feeding 2–5–25.

2–26—473000..... 2/23—56 min.
3–5 —148000..... 3/3 —77 min.
Hemorrhage 3–3. Treated. No sweet clover until 4–1–25.
3–10—535000..... 3/19—60 min.
3–27—570000..... 17 min.
4–6 —..... 20 min.
4–9 —377000..... 4/13—22 min.
4–22—455000..... 4/20—52 min.

No. 9842
Started feeding 3–9–25

3–10—513000..... 27 min.
3–27—510000..... 1 hr.
4–8 — 81700..... 4/6—1 hr.
Hemorrhage 4–7–25. Treated, recovered no sweet clover until 4–20.
4–9 —130000..... 24 min.
4–14—713000..... 4/13—33 min.
4–23—380000..... 4/20—14 min.
5–4 —480000..... 5/5—2 hrs.+
5–12—517000.....
5–16—395000..... 5/8—80 min.
Hemorrhage 6–15–1925.

No. 9841
Started feeding 3–9–25

3–10—350000..... 3/12—39 min.
3–27—459000..... 57 min.
4–9 —337000..... 4/6 —55 min.
4–14—395000..... 4/13—1½ hr.
Dead—4/19/1925.

Hemorrhage first observed 4–22.
Clotting time 4/23—2 hrs. Treated, recovered.
4–24—156000.....
4–25—162000..... 35 min.
4–27—410000..... 27 min.
5–5 —568000..... 4/28—35 min.

It is interesting to report that no. 9750 developed the characteristic hemorrhage on three successive occasions. The third feeding experiment resulted fatally. This condition does not result in immunity

Seven counts made on three additional calves at times when the bloods required intervals in excess of 56 minutes for coagulation gave counts varying from 212,000 to 551,000 per cubic millimeter.

This work failed to demonstrate a diminution in the platelets which would explain the failure of coagulation but rather suggested an occasional increase. When animals were treated and recovered from the effects of the hemorrhage, the platelets reappeared in increased numbers. The hematocrit method of Van Allen (1926) was then used to determine the percentage of platelets present during a series of nine feeding experiments. Part of the results follow with the progressive delay in coagulation for comparison.

No. 76. Started feeding 2–1–1928

	DATE														
	2/6	2/8	2/10	2/13	2/15	2/17	2/20	2/22	2/24	2/27	2/29	3/2	3/5	3/6	3/7
Per cent	0 35	0 375	0 49	0 51	0 54	0 51	0 42	0 48	0 45	0 38	0 45	0 58	0 40	0 36	*
Time	7	—	21	—	22	20	33	51	45	89	63	76	50	—	†

* Hemorrhage 3/6.
† Not in 2 hrs.

No. 77. Started feeding 2–2–28

	DATE																
	2/3	2/6	2/8	2/10	2/13	2/15	2/17	2/20	2/22	2/24	2/27	2/28	2/29	3/2	3/10	3/12	3/14	3/16
Per cent	0 30	0 30	0 37	0 375	0 42	0 46	0 48	0 48	0 38	0 50	0 60	0 47	0 34	0 40	0 30	0 62	0 75	0 925
Time (min.)	—	6	—	20	—	18	21	29	48	51	106	—	55	—	25	—	11	25

Hemorrhage 3–2–28 Treated. Recovered.

No. 78. Started feeding 3–7–28

	DATE													
	3/7	3/9	3/12	3/14	3/16	3/19	3/21	3/23	3/26	3/28	3/29	3/30	4/2	4/4
Per cent	0 54	0 54	0 57	0 70	0 60	0 47	0 61	0 53	0 50	0 42	0 48	0 50	0 32	0 175
Time	21	—	25	12	21	24	28	45	43	120	—	135	75	*

* Did not clot. Hemorrhage 4–5–28.

No. 79. Started feeding 3-7-28

	DATE													
	3/7	3/9	3/12	3/14	3/16	3/19	3/21	3/23	3/26	3/28	3/29	3/30	4/2	4/4
Per cent	0.35	0.375	0.52	0.56	0.57	0.565	0.60	0.51	0.36	0.15	0.20	0.10	0.17	0.25
Time	23	—	18	44	—	33	96	34	50	102	—	99	138	*

* Failed. Dead of internal hemorrhage 4-7-28.

The progressive decrease in the quantity of available prothrombin seems to be quite independent of the number of platelets present. The drop in platelets at or near the time of visible hemorrhage may be another evidence of the injury which results in the hemorrhage.

TREATMENT. The writer found that the intravenous injection of freshly defibrinated bovine blood from a normal animal which was not fed on sweet clover resulted in the prompt recovery of many animals which already showed the effects of serious hemorrhage. Quantities of 500 cc. or, better, one liter of blood appear adequate for cattle weighing 250 to 500 pounds. The results are equally favorable whether the blood is injected within 10 minutes after it is drawn or if several hours elapse before the transfusion. Normal citrated blood is likewise effective. The injection of the defibrinated blood controls the hemorrhage by a prompt but somewhat temporary improvement in the clotting time. A change of feed then permits recovery. A possible explanation of this phenomenon may be on the basis of the prothrombin content of the citrated blood. Mills (1926) found that the thrombin disappears in serum and prothrombin is regenerated so that this reaction may occur in the defibrinated blood so that the deficient prothrombin is temporarily replenished in the circulating blood when the transfusion is performed.

SUMMARY

The feeding of damaged sweet clover hay and silage results in losses whereby the animals die of hemorrhage which is preceded by a characteristic delay in the coagulation of the blood. This paper deals with a consideration of the alterations in the blood which are concerned in the coagulation process.

Analytical work on the blood of a series of animals fed on damaged sweet clover indicates that there may be some depression in the blood fibrinogen during the feeding trials yet the values obtained were within the probable error of a series of determinations on normal cattle.

This work shows that the delayed coagulability of the blood in sweet clover disease involves a reduction in the prothrombin and that the diminution parallels the delay in the coagulation time.

No experimental evidence has been found to indicate that the delay in the coagulation time is due to the increase of an inhibitory principle of the nature of heparin or antithrombin. Estimation of the blood calcium together with other studies show that this condition is neither caused by nor does it involve a deficiency in the metabolism of calcium.

The progressive decrease in the quantity of available prothrombin in the blood of animals fed on the damaged sweet clover seems to be quite independent of the number of platelets present which remains at about the normal level until hemorrhage occurs.

The treatment of many animals which have shown the effects of serious hemorrhage by the intravenous injection of freshly defibrinated normal bovine blood has resulted in a large proportion of recoveries.

BIBLIOGRAPHY

Bayne-Jones, S. 1912. This Journal, xxx, 74.

Bordet, J. 1921. Bull. Johns Hopkins Hosp., xxxii, 213.

Cekada, E. B. 1926. This Journal, lxxviii, 512.

Clark, G. W. 1921. Journ. Biol. Chem., xlix, 487.

Duke, W. C. 1928. Journ. Amer. Med. Assoc., xci, 720.

Foster, D. P. and G. H. Whipple. 1921–22. This Journal, lviii, 407.

Gram, H. C. 1921. Journ. Biol. Chem., xlix, 279.

Hart, E. B., H. Steenbock and G. C. Humphrey. 1920. Wis. Agric. Exp. Sta. Research Bul. no. 49.

Hart, E. B., H. Steenbock, C. A. Hoppert and R. M. Bethke. 1922. Journ. Biol. Chem., liv, 75.

Howell, W. H. 1914. Arch. Int. Med., xiii, 76.
1926. This Journal, lxxvii, 680.

Howell, W. H. and E. B. Cekada. 1926. This Journal, lxxviii, 500.

Hurwitz, S. H. and C. K. Drinker. 1915. Journ. Exper. Med., xxi, 401.

Larrabee, R. C. 1920. Journ. Amer. Med. Assoc., lxxv, 1632.

Meigs, E. B., N. R. Blatherwick and C. A. Cary. 1919. Journ. Biol. Chem., xxxvii, 1.

Mills, C. A. 1926. This Journal, lxxvi, 632.
1927. Chinese Med. Journ., i, 235.

Robinson, C. S. and C. F. Huffman. 1926. Journ. Biol. Chem., lxvii, 245.

Roderick, L. M. 1929. Journ. Amer. Vet. Med. Assoc., lxxiv, 314.

Roderick, L. M. and A. F. Schalk. 1931. Studies on sweet clover disease. Bull. N. D. Agric. Exp. Sta.

Rosenthal, N. 1928. Journ. Lab. Clin. Med., xiii, 303.

Schofield, F. W. 1924. Journ. Amer. Vet. Med. Assoc., lxiv, 553.

Van Allen, C. W. 1926. Journ. Lab. Clin. Med., xii, 233.

HEMODYNAMICS OF ARTERIOSCLEROSIS

Influence of Elastic Factor on Circulation

GEORGE FAHR, JAY DAVIS AND RUSSEL SPITTLER

From the Department of Medicine, University of Minnesota

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Arteriosclerosis may be defined as an increase in the rigidity or the coefficient of volume elasticity of the arteries in so far as the circulation of the blood in the arteries is concerned. In addition there is usually a change in the diameter of the lumen of the vessels in arteriosclerosis. The mean diameter of the lumen of the vessels enlarges on the one hand due to stretching of the walls, and on the other hand there is a tendency to narrowing of the lumen due to thickening of the intima. Usually the large vessels in arteriosclerosis are wider than in the normal state and the small arteriosclerotic vessels are narrower. Narrowing would tend to cause high blood pressure and widening would tend to cause a lowering of blood pressure.

In this paper we shall not consider these static changes in lumen. There will be two principal factors to be considered in studying the effects of arteriosclerosis upon the circulation of the blood if we confine our investigation to the case where the lumen of the arterial system is the same in the normal and in the arteriosclerotic state for the same diastolic blood pressure. The first factor is the greater increase in lumen during systole in the normal arterial system. In other words, resistance to blood flow is less in the normal due to greater increase in diameter during systole and the early part of diastole. I doubt if the decrease in resistance to blood flow due to this factor is greater than 10 per cent of the total resistance to flow in the human vascular system. In this paper we shall neglect this factor and investigate only the influence of the elastic factor itself or the wind chamber effect of various degrees of rigidity upon the work of the heart and the blood flow through the arteries.

We (1) have pointed out that the external work of the left ventricle is probably very slightly increased in arteriosclerosis provided the "mean" blood pressure is kept constant and the stroke output remains the same. This makes it highly improbable that a work hypertrophy of the left ventricle is produced in generalized arteriosclerosis even of high degree if the blood pressure is not elevated at the same time. Our conclusions

were arrived at through a consideration of the well known work integral for the heart and A. V. Hill's (2) curve of volume elasticity for the human arterial system. These conclusions arrived at analytically should be tested experimentally especially as they were made without a knowledge of the time relations of the "run off" from the arterial system to the venous system. Moreover the hemodynamics of arteriosclerosis are of sufficient practical importance to deserve a much more thorough experimental study than has hitherto been made. This paper is a preliminary study of the hemodynamics of arteriosclerosis, to discover the effect of increasing the coefficient of volume elasticity in an arterial system whose "resistance" or "effective" bore remains essentially the same both in the condition where the coefficient of volume elasticity for the arterial system is small, as well as in the conditon where the coefficient of volume elasticity is very large and therefore approximates the condition in severe arteriosclerosis. In other words, this is an investigation of the wind-chamber effect or elastic factor upon the circulation apart from lumen changes.

The heart-lung preparation and apparatus of Starling modified as in figure 1 was used for the investigation. In the earlier experiments the arterial cannula, lumen 4.5 mm., was inserted into the brachiocephalic artery. A larger arterial cannula, internal diameter 9 mm., was inserted in the later experiments through the thoracic aorta up close to aortic valves, at the suggeston of G. V. Anrep, who pointed out that we were working with what was essentially an aortic stenosis, when the smaller cannula was inserted into the brachiocephalic artery and the whole stroke volume was pumped through it. Most of our experiments have been made with the large cannula. Dogs of 18 to 30 pounds were used. The arterial system was glass excepting where parts were connected by rubber tubing. The glass connections were very closely approximated so that essentially the arterial system as well as the manometer system attached to the arterial cannula were of glass. The coiled glass tube which represents the large artery system was 10 mm. in internal diameter, had a length of 120 cm. from the aortic cannula to the artificial resistance apparatus and an internal volume of 92 cc. The aortic cannula had an internal volume of 20 cc. The artificial resistance was made of thin walled rubber tubing surrounded by a small air chamber. This part of the arterial system had the smallest rigidity. A glass tube 10 cm. long and internal diameter 3.5 mm. led from the arterial system into the artificial resistance. The fall of pressure along this small tube and the artificial resistance is very much greater than the fall of pressure along the rest of the arterial system, as it should be if this part of the system is to represent the arterioles and capillaries of the animal.

The resistance of the arterial system from the aortic cannula to the

beginning of the artificial resistance was found to be about one-half the resistance of the artificial resistance system at very low blood pressures. The resistance of the system from the aortic cannula to the artificial resistance system was 22 per cent of the resistance from the aortic cannula to the end of the artificial resistance at medium blood pressures. When

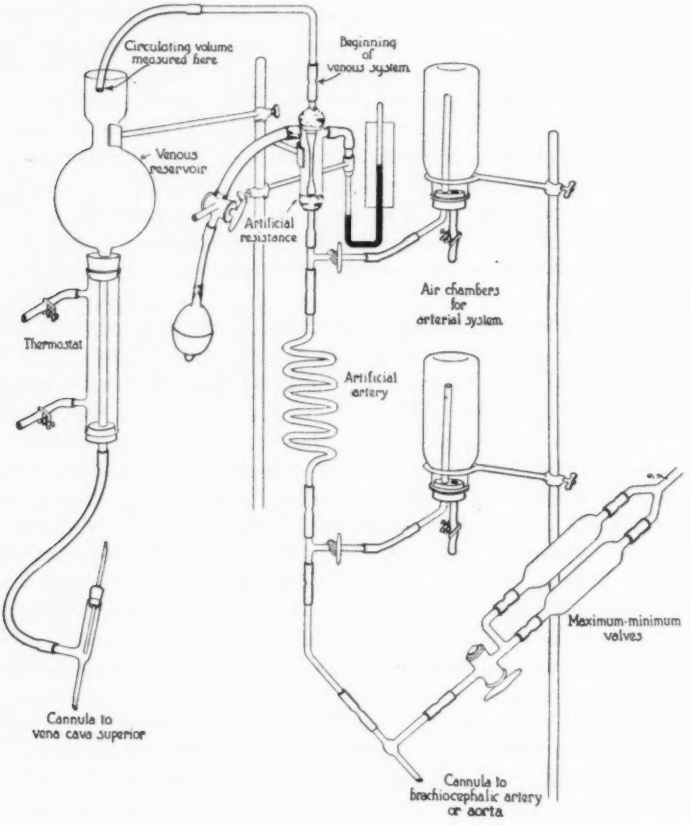

Fig. 1. Artificial arterial and venous system connecting aorta with vena cava superior.

the artificial resistance was compressed to such a degree as to give very high blood pressures in the aorta, the resistance of that part of the system from the aortic cannula to the artificial resistance was found to be 12 per cent of the total resistance to flow from aortic cannula to venous end of the artificial resistance.

Two air chambers each of 1000 cc. capacity were connected through one way cocks with the arterial system as in figure 1. When these cocks were closed the arterial system, i.e., the glass tubing from the arterial cannula mouth to just beyond the artificial resistance and including the manometer system, was of great rigidity, the coefficient of volume elasticity for this system being about 10 times greater than that for A. V. Hill's (3) "mean" normal artery at pressures between 80 to 120 mm. Hg and about 3 times greater than the greatest rigidity found by Hill in an arteriosclerotic man. The rigidity of the system as measured by the coefficient of volume elasticity was about one-half that for Hill's normal artery when the cocks were opened and the arterial system was in free communication with the two bottles. Therefore the rigidity of the arterial system was 20 times as great without air chambers as with air chambers.

A maximum-minimum manometer was used to measure the blood pressure in most of the experiments because there was no elastic manometer of sufficiently high free period of vibration at our disposal when we started the investigation and we considered it more advisable to get fairly accurate systolic and diastolic pressures than to get curves with an ordinary mercury manometer whose values might have very little relation at all to the true pressures especially in the arteriosclerotic system. A Frank elastic manometer as modified by Broemser with a free period of 40 per second was used in the last experiments.

The circulating minute volume, i.e., the true minute volume minus the coronary flow was measured by means of a stop watch graduated to 0.1 second and measuring cylinder. Blood leaving the end of the rubber tubing and entering the venous reservoir could be diverted into the cylinder at will. The time taken to fill the cylinder between two marks upon it could be determined and in this way the minute flow of blood determined with an accuracy of ± 3 per cent.

The stroke volume was determined from cardiometer tracings. A Henderson cardiometer connected to a segment tambour of Wiggers was calibrated after each series of experiments by means of a record syringe attached to the cardiometer transmission line. Twenty cubic centimeters of air were injected and the corresponding fall in the tambour lever determined upon the kymograph drum. The error in the measurement of stroke volume was not determined. In some of the latter experiments where we had grown careless in the application of the cardiometer around the atrioventricular ring, the error in the measurement of stroke output may have been considerable. We attempted to determine the minute flow, through the coronary arteries, by subtracting the relatively accurate circulating minute volume from the true minute volume as determined from one-half the cardiometer stroke volume times the heart rate. The

values for coronary flow thus determined very probably show the order of magnitude of the coronary flow fairly well in most experiments, but the absolute values are probably inaccurate and in one experiment the error in the measurement of stroke output is so great as to give an apparent negative coronary flow. On the other hand the diastolic position (lower position in our curves) of the cardiometer remained constant for long periods of time provided nothing was altered in the filling or the output of the heart or in the pressure in the aorta.

Those periods of an experiment when the air chambers were connected to the arterial system were marked upon the kymograph paper by a signal magnet. Time was measured in seconds by a Jacquet chronometer.

The coefficient of volume elasticity was determined for the arterial system with air chambers attached and with air chambers detached as follows: The arterial cannula was removed from the heart and connected to a record syringe filled with saline. The whole system was kept filled with blood. Just beyond the artificial resistance a cork was placed over the glass tube emerging from the artificial resistance thus closing the system at this end. From the record syringe 2 cc. amounts of saline were injected into the arterial system without air chambers, and 20 cc. amounts of saline were injected into the arterial system with air chambers connected. The corresponding rises in arterial pressure were recorded by the mercury manometer of the maximum-minimum manometer system. The fluid volume in the whole system with and without air chambers and including the manometer system was measured. The percentage increase in fluid volume could then be calculated and equated to the pressure rise. We have expressed rigidity or volume elasticity in terms of 1 per cent increase in volume to corresponding rise in millimeters mercury pressure. There is one very small error in our determinations of coefficient of volume elasticity, namely, the small portion of aorta, 1.5 to 2 cm., from the root of the aorta to the tip of the aortic cannula. This is not accounted for in the calibration, as the aortic cannula is removed from the aorta and the syringe attached to it for calibration. All branches of the aorta were tied close to the arch so that only this small portion of the ascending aorta which is not in the system during calibration may be a source of very slight error in the determination of the coefficient of volume elasticity remained.

The heart-lung preparation of Starling, which was the pumping system used by us, when properly made is a most excellent means for studying questions such as these. In some of our experiments the heart did not dilate perceptibly, i.e., 1 cc. within two hours while doing a large amount of external work.

In drawing conclusions as to the work of the heart from our experiments, we consider as well established by Starling and Visscher (4) that the diastolic volume of the heart is an index of the total energy consumption

of the heart. It would therefore seem very probable that the diastolic volume of the heart or the length of the muscle fibres determines the external work of the heart provided the heart muscle function and mechanical efficiency of the heart remain constant throughout the period under consideration. Frank (5) and Starling (6) believe that their work proves that the diastolic volume of the heart is determined by the factors of pressure and minute volume, which factors determine the external work of the heart. Most physiologists accept this work as conclusive especially as it is in agreement with the facts of smooth muscle physiology. In determining the external work of the heart physiologists have used the product of the minute volume by the arithmetical mean pressure. This is not an accurate measure of the external work of the heart which is given by the integral $\int Pdv + \frac{1}{2} Mv^2$ but is probably a very good first approximation. No one has ever measured accurately the external work of the heart because the integration can only be carried out when accurate minute volume curves and pressure curves are recorded. In a subsequent paper we hope to record optically the necessary data for determining the external work of the heart accurately. For the present we shall content ourselves with the same approximation that most physiologists have used, namely, the product of minute volume by arithmetical mean pressure, the minute volume being measured by a cardiometer and the blood pressure by a maximum-minimum manometer.

The heart will dilate if the external work remains the same but the functional condition is injured by some agent or factor. Likewise the heart will get smaller doing a given amount of work if the functional condition is improved by some agent or factor.

If such agents or factors are ruled out, then an increase in diastolic volume of the heart means increased external work and a reduction in diastolic volume means decreased external work. Unchanged diastolic volume means unchanged amount of external work performed by the heart.

This work was done with an open venous system, i.e., an extremely small coefficient of volume elasticity for the venous system. We also experimented with a closed system in which the rigidity of the venous system was regulated by an air chamber to $\frac{1}{5}$ the rigidity of the arterial system with chambers connected. This air chamber could be opened at will and thus an open venous system established. The minute volume, diastolic volume, blood pressure and systemic volume flow remained the same to within 5 per cent, using the open or closed venous system. We therefore concluded that our results could be applied to the mammalian circulation, because the rigidity of the veins is very much smaller than that of the arteries. The work will be repeated in the future using various degrees of rigidity in the venous system. We believe that our conclusions can

be directly applied to the condition of arteriosclerosis in man because of the large volume of the venous system and the comparatively small degree of rigidity of the veins.

Experiment of October 15, 1929. Calibration of elastic coefficient in terms of percentage volume increase of arterial system to millimeter mercury increase in pressure or

$$\frac{\text{mm. Hg increase in pressure}}{\text{\% increase in volume}} = \text{"effective" elastic coefficient or rigidity of arterial system.}$$

Without air chamber (arteriosclerosis)

Content of "effective" arterial system = 210 cc.

2 cc. fluid injected into system gives a rise of 58 mm. Hg in pressure within this system.

$\frac{2}{210}$ = 0.95 per cent increase in volume for 58 mm. Hg

or

1 per cent increase in volume for 61 mm. Hg

A. V. Hill's normal artery between 80 — 120 mm. Hg showed 1 per cent increase in volume for 6 mm. Hg.

In this arterial system without air chambers and representing extremely severe arteriosclerosis, there is a rigidity 10 × "mean" normal artery of A. V. Hill at normal pressure.

With air chamber (normal)

Content of arterial system = 240 cc.

20 cc. fluid injected into arterial system gives a rise of 32 mm. Hg

$\frac{20}{240}$ = 8.3 per cent increase in volume for 32 mm. Hg pressure

or

1 per cent increase for 3.9 mm. Hg increase in pressure

The rigidity is $\frac{3}{4}$ of A. V. Hill's "mean" normal artery between the pressures of 80 — 120 mm. Hg.

Rigidity without air chamber (arteriosclerosis) = 15½ times rigidity with air chambers (normal).

Arterial cannula in brachiocephalic artery ø = 4.5 mm. At narrowest point ø = 3 mm. In this experiment all points in arterial system beyond the bulb of cannula where manometer arm goes off were 8 mm. or more in diameter. There was the equivalent of an aortic stenosis but no abnormally high resistance in arterial system beyond the narrow part of cannula.

Calibration of cardiometer

20 cc. = 16 mm.

With air chambers (Normal)			*Without air chambers (Arteriosclerosis)*	
Sys. B. P.	122	Mean B. P.	126	Mean B. P.
Diast. B. P.	72	97	64	95
Stroke Vol.	7.5 cc.	Rate of heart 125	7.5 cc.	
Minute Vol.	937 cc.		937 cc.	
Circul. Vol.	857 cc.		874 cc.	
Coronary flow	80 cc.		62 cc.	

Other measurements made in course of this experiment show similar results.

Minute Vol. = Total output of left ventricle calculated from ½ cardiometer change in systole multiplied by heart rate.

Circul. Vol. = Volume of flow as measured with stop watch and calibrated measuring cylinder on blood flowing out of venous system into venous reservoir.

Stroke Vol. = Output of one ventricle. This value is obtained by multiplying the height of the cardiometer curve in millimeters by 20 and dividing by 2 times the increase in height of the cardiometer tracing when 20 cc. are forced into the cardiometer system from a record syringe connected to the system.

When arterial system was very rigid, i.e., without air chambers, the blood spurted from the end of the rubber tubing into the venous reservoir in jets. The flow was very steady with the air chambers connected.

Heart was dilating continuously from start of this set of experiments. Apparently no difference in diastolic volume without air chamber and with air chamber in arterial system.

We interpret this experiment as demonstrating that the energy consumption of the heart remained unchanged when the coefficient of volume elasticity of the arterial system is increased 15 times because the diastolic volume remained constant and that the external work of the left ventricle as a first approximation remained the same after increasing the coefficient of volume elasticity 15 times.

Venous inflow increased! and heart dilated for about 20 beats, then got smaller with this increased flow showing need for coronary circulation. At end of 30 beats was about 2.5 cc. smaller than before increase of inflow:

	Normal			*Arteriosclerosis*	
Sys. B. P.	129	Mean B. P.		136	Mean B. P.
Dias. B. P.	82	106		72	104
Stroke Vol.	9.4 cc.			9.4 cc.	
Minute Vol.	1175 cc.		Rate of heart 125	1175 cc.	
Circul. Vol.	1077 cc.		3 similar experiments	1077 cc.	
Coronary flow	98 cc.			98 cc.	

In normal (with air chamber) diastolic volme is 1.0—1.5 cc. larger than in arteriosclerosis (without air chamber) in 3 experiments carefully measured.

Venous inflow again increased! Heart dilates but recovers all but about 1.5 cc. of dilatation.

Sys. B. P.	140	Mean B. P.	148	Mean B. P.
Dias. B. P.	90	115	78	113
Stroke Vol.	11.3 cc.		11.3 cc.	
Minute Vol.	1532 cc.		1532 cc.	
Circul. Vol.	1312 cc.		1312 cc.	
Coronary flow	220 cc.		220 cc.	

Heart is very slowly dilating for next ½ hour of experiment. It is a slow continuous dilatation of 4 cc. in ½ hour at an even rate of dilatation throughout. In these 3 experiments the diastolic volume of heart in arteriosclerosis (without air chamber) is 0.75 to 1.0 cc. smaller than in normal (with air chamber).

In these experiments the diastolic volume of the heart was slightly less (2 per cent) with an increase in rigidity of the whole system than when the coefficient of volume elasticity was $\frac{1}{15}$ that of the more rigid system; in other words a slightly smaller total energy output of the heart when working against the system without air chambers. The external work of the left ventricle is very apparently not greater when working against the more rigid arterial system. The minute volume remains constant and the mean B. P. falls in the rigid system. It is possible that the lowering of the mean

and diastolic blood pressures is the cause of a slight decrease in external work of the left ventricle when working against the system without air chambers.

Experiment of April 16, 1929. Calibration of elastic coefficient in terms of percentage volume increase of arterial system to millimeter mercury increase in pressure.

Without air chamber (arteriosclerosis)

1 per cent increase in volume for 40 mm. Hg

A. V. Hill's normal artery between 80 — 120 mm. Hg showed *1 per cent* increase in volume for *6 mm. Hg*

In this system without air chambers and representing extremely severe arteriosclerosis, there is a rigidity 6.6 × normal "mean" artery of A. V. Hill at normal pressure.

With air chamber (normal)

1 per cent increase for 2 mm. Hg.

The rigidity is $\frac{1}{3}$ that of A. V. Hill's "mean" normal artery.

Rigidity without air chamber or in arteriosclerosis = 20 × that with air chambers or in normal.

Cannula in brachiocephalic artery ϕ = 4.5 mm. at mouth. ϕ = 3 mm. at narrow point. Hence very marked aortic stenosis is present. There is also a very high resistance in the form of a short glass tube of very narrow bore where blood enters artificial resistance in this experiment.

Calibration of cardiometer

20 cc. = 16 mm.

Sys. B. P.	224	191		246	186
Dias. B. P.	158			126	
Stroke Vol.	12.7			12.2	
Minute Vol.	1270		Rate of heart 100	1220	
Circul. Vol.	833			857	
Coronary flow	437			363	

Many other measurements of this day's experiment show similar results.

The diastolic volume of the heart in the experiments with air chambers in the arterial system (normal) was approximately 1.5 cc. larger than in the experiments with great rigidity of the system. We interpret these experiments as demonstrating that the total energy consumption of the heart is not increased when under conditions of large minute volume and high blood presure the heart works against an arterial system of 20 times the previous volume rigidity due to absence of air chambers. They also demonstrate to a first approximation that the external work of the left ventricle is not increased when going over to the more rigid system. It will be noted that whereas the minute volume remains nearly constant when working against the system without air chambers, the mean blood pressure has a tendency to fall. Perhaps the fall in mean and diastolic blood pressure tends to reduce the external work of the left ventricle very slightly.

Experiment of October 22, 1929. Calibration of elastic coefficient in terms of percentage volume increase of arterial system to millimeter mercury increase in pressure.

Without air chamber (arteriosclerosis)

1 per cent increase in volume for 59 mm. Hg.

A. V. Hill's normal artery between 80 to 120 mm. Hg showed *1 per cent* increase in volume for *6 mm. Hg.*

In this system without air chambers and representing extremely severe arteriosclerosis, there is a rigidity 9.8 × normal "mean" artery of A. V. Hill at normal pressure.

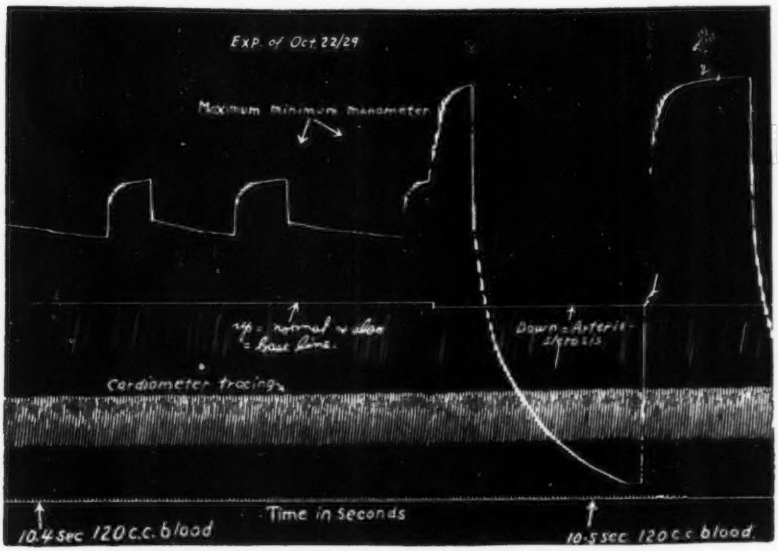
Exp. of Oct 22/29
Maximum minimum manometer
Up = normal + above = base line.
Down = Arterio-sclerosis
Cardiometer tracing
Time in seconds
10.4 sec 120 c.c. blood
10.5 sec 120 c.c. blood.

Fig. 2. Kymograph tracing on day when diasolic pressure in the aorta during period of increased rigidity was negative. Quite recently, making use of an ordinary mercury manometer without maximum-minimum valves, we have recorded negative diastolic pressure in the condition of great rigidity of the arterial system. Upstroke of cardiometer tracing is systole, downstroke diastole.

With air chamber (normal)

1 per cent increase for 3.3 mm. Hg

The rigidity is ½ that of A. V. Hill's "mean" normal artery.

Rigidity without air chamber or in arteriosclerosis = 18 × that with air chambers or in normal.

Aortic cannula $\phi = 9$ mm. except at the narrow point where $\phi = 8$ mm. Cannula in arch of aorta at a point about 1½ cm. from valves. *No aortic stenosis now present nor in any of the subsequent experiments.*

Calibration of cardiometer

20 cc. = 13 mm.

With air chamber (Normal)			*Without air chamber (Arteriosclerosis)*
Sys. B. P. = 80 } 61 mm. Hg			140 } 12? mm. Hg
Dias. B. P. = 42 }			116 }
Stroke Vol. = 13.0 cc.	Rate of heart 78		13.0 cc.
Minute Vol = 1014 cc.			1014 cc.
Circul. Vol. = 686 cc.	Other measurements made		692 cc.
Coronary flow = 328 cc.	in course of 2 hours show similar results.		322 cc.

Diastolic volume is 0.75 cc. less without air chambers than with air chambers.

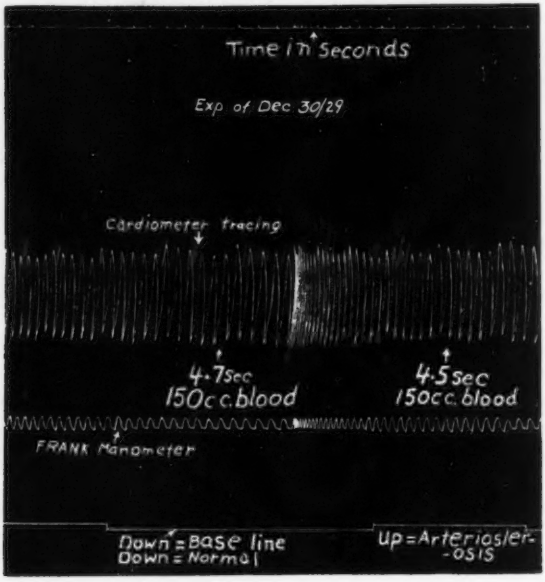

Fig. 3. Kymograph tracing taken on a day when Frank elastic manometer was used for recording blood pressure rather than mercury manometer with maximum-minimum valves. Upstroke of cardiometer tracing is systole; downstroke diastole.

Throughout course of experiment lasting 2 hours heart volume in diastole is approximately 3 cc. less at end than at beginning of experiment: It will be noted that diastolic blood pressure in arteriosclerosis is negative in this series of experiments. We have no explanation for this. There seems to be no question about the negativity as figure 2 shows.

These experiments demonstrate that the total output of energy by the heart is not increased, indeed seems to be slightly decreased when working against an arterial system without air chambers and with a coefficient of volume elasticity 18 times greater. The external work of the left ventricle is as a first approximation not in-

creased but rather decreased because the heart is smaller. This decrease in external work of the left ventricle is very small and if outside the limits of error of the method is probably due to the decrease in mean blood pressure consequent upon the great fall in diastolic pressure.

Experiment of December 30, 1929. This experiment was performed with a Frank elastic manometer as blood pressure recording instrument instead of maximum-minimum mercury manometer.

Calibration of elastic coefficient in terms of percentage volume increase of arterial system to millimeter mercury increase in pressure.

With air chamber (normal)

1 per cent increase for 4.3 mm. Hg

The rigidity of this arterial system is $\frac{2}{3}$ or 70 per cent of A. V. Hill's "mean" normal artery between the pressure 80 to 120 mm. Hg

Without air chamber (arteriosclerosis)

1 per cent increase for 53 mm. Hg rise in pressure.

The rigidity of this arterial system without air chamber connections is 9 times the rigidity of A. V. Hill's normal artery and 13 times the rigidity of the same arterial system with air chambers connected to system.

Calibration of Frank manometer

Free period = 40 cycles per second
1 mm. rise of curve = 27.5 mm. Hg
Zero point of Frank manometer = 27.5 mm. above lowest signal line:
Calibration of cardiometer 20 cc. = 19 mm. Cardiometer probably not accurately applied in this experiment.

	With air chamber (Normal)		*Without air chamber (Arteriosclerosis)*	
Systolic B. P.	206	161	220	152
Diastolic B. P.	116		83	
Circulating Vol.	1920 cc.		1990 cc.	
Stroke Vol.	15.1 cc. ?		14.4 cc. ?	
Rate of Heart	100		100	
Minute Volume	1510 cc.?		1440 cc. ?	
Coronary Flow				

Diastolic volume (mean of ten diastoles) is 0.8 cc. less without air chamber than with air chamber (mean of ten diastoles). In other words, no increase in volume but rather a very slight decrease in volume in the condition of increased rigidity. Cardiometer values for stroke volume of heart are much too low. Probably due to improper position of cardiometer on the heart. Minute volume must be about 2400 cc. This heart is doing nearly as much work as the heart of an athlete during severe exertion. The minute volume as well as the blood pressure are increased far beyond the normal. Even under these circumstances the heart does not dilate during the period when it is pumping through the rigid system nor is the volume of the systemic circulation reduced. We believe that this experiment proves that even under conditions of nearly maximum work of the left ventricle the total energy output of the heart when working against a rigid system is not increased provided the effective lumen of the system remains the same. The external work of the left ventricle when measured by diastolic size seems to remain the same against the rigid system. The external work of the left ventricle as measured by the product of minute volume and mean blood pressure seems to be less against the more rigid system probably largely because the mean blood pressure is lower.

These protocols demonstrate that there is no dilatation of the heart when the air chamber is removed provided there is no change in the effective lumen of the artierial system in changing from the more extensible to the more rigid system and provided the extensibility of the venous system is much greater than the extensibility of the arterial system. This was true whether there was an aortic stenosis present or not, whether the blood pressure was high or low or the minute volume small or large. If we accept the modern theory of the relation between diastolic volume and total energy consumption of the heart and agree that Starling and Frank have demonstrated to at least a first approximation that the diastolic volume of the heart is a measure of the external work of the heart, then we believe that we are justified in drawing the following conclusions from our experimental work.

CONCLUSIONS

Using the heart-lung preparation of Starling with an artificial arterial system of such a nature that changing the coefficient of volume elasticity does not materially alter the lumen of the system, and making use of an open venous system or a closed venous system of very low coefficient of volume elasticity, both with and without artificial aortic stenosis, both with low and with very high blood pressures and with small as well as with large heart outputs, we find that

1. The total energy consumption of the heart is not increased by increasing the coefficient of volume elasticity many times (10) the coefficient of volume elasticity of the normal human arterial system.

2. The external work of the left ventricle to at least a first approximation is not increased by increasing the coefficient of volume elasticity many times that of the normal human arterial system.

3. That the circulatory volume flow is not decreased by increasing the coefficient of volume elasticity many times (10) the normal.

4. That the coronary flow is usually adequate but perhaps somewhat reduced when the rigidity of the arterial system is increased beyond the normal.

5. That the diastolic pressure falls more than the systolic pressure rises in artificial arteriosclerosis so that the arithmetical mean pressure is somewhat less so long as the peripheral resistance remains the same.

6. That the stroke output remains practically constant when the wind chamber effect of the elasticity of the arteries is removed.

7. That the wind chamber effect steadies the volume flow in the arteries but has very little other value in short experiments extending over an hour or two.

BIBLIOGRAPHY

(1) Fahr, G. Proc. Soc. Exper. Biol. and Med., 1927, xxiv, 405.
(2) Hill, A. V., J. C. Brammell and A. C. Downing. Heart, 1923, x, 289.
(3) Hill, A. V. Loc. cit.
(4) Starling, E. H. and M. B. Visscher. Journ. Physiol., 1927, lxii, 243.
(5) Frank, O. Zeitschr. f. Biol., 1895, 372.
(6) Starling, E. Linacre Lecture, 1916.

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

I. Respiratory Metabolism and Certain Changes in the Blood of Anesthetized Dogs

E. S. NASSET, F. W. BISHOP and S. L. WARREN

From the Department of Vital Economics, University of Rochester, and the Department of Radiology of the University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

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The generation of heat in the living animal body by the passage of high frequency electric current was early described by d'Arsonval (1893). The path taken by this type of current and the relation of the circulation of blood to local heating has been investigated by Binger and Christie in a series of excellent experiments (1927, 1928). Bettman and Crohn (1927), Lonergan (1927), Nagelschmidt (1926) and others have contributed to the knowledge of the action of high frequency current when passed through living and dead animal tissues. The literature dealing with the different responses of the animal body to other sources of heat has been reviewed by Bazett (1927).

The high frequency current has been in use clinically for about twenty-five years. Nagelschmidt (1926) introduced the term "diathermy," *i.e.*, heating through, to designate the usage of high frequency current for treatment purposes. The terms, "diathermy," and "heating by high frequency current," are used interchangeably in this paper. In spite of its quarter century of clinical application, comparatively little is known about the effects of diathermy upon normal metabolic processes. Durig and Grau (1913) studied the respiratory exchange before, during and after treatment of three normal subjects with high frequency current. They were interested in determining whether the body might use the heat thus obtained to supplant some of the heat of metabolism and so reduce the actual energy output of the organism. In each case they found an increased oxygen consumption, even with moderate elevations in temperature (up to 1.1°C.). According to Stary (1926), who experimented with normal and anesthetized rabbits, small doses of diathermy seemed to markedly reduce the oxygen consumption of normal rabbits. He maintained that the urethane narcosis employed in the other animals eliminated the regulatory activity of the heat center and that in these cases the high

frequency current increased the metabolism. It is unfortunate that he did not describe his work in detail. Bischoff and co-workers (1930) reported increases in the metabolic rate of two normal and two cancerous human subjects following treatment with diathermy. Their work was concerned chiefly with blood chemistry studies and includes only a single metabolism determination in each case following application of the current.

We have attempted to get a series of determinations of the progressive changes in metabolism occurring in anesthetized dogs during treatment with diathermy. Temperatures were simultaneously measured in several different regions of the body. The number of experiments upon which this paper is based is not large but in view of the uniformity of the gross changes noted, we feel justified in making a report at this stage of our investigation. It is planned eventually to study rather extensively both normal and anesthetized animals as well as hospital patients and normal human subjects. A preliminary report of this work has been made (Nasset and Warren, 1930).

PLAN OF THE EXPERIMENT. The dogs used in the experiments reported in this paper were mongrels, weighing 9 to 27 kgm., obtained from stock. They were fasted at least 18 hours. Morphine sulfate (0.015 to 0.030 gram) was injected subcutaneously 30 to 60 minutes prior to the intravenous administration of 35 to 45 mgm. per kilogram of sodium amytal (Lilly). Connection to a Benedict Universal respiration apparatus was made with the trachea by means of a glass cannula. The distance from the site of tracheotomy to the air circuit was never greater than to the tip of the nose. Oxygen consumption was measured by a "wet" gas meter the accuracy of which was found to be $\pm$ 2 per cent for 2 liters of gas. Ordinarily the amount of oxygen consumed in each metabolism period exceeded this quantity. The average of nine alcohol checks made during this investigation was 0.670. A writing point attached to the spirometer of the metabolism apparatus recorded the respiratory movements on a long smoked paper. This record also served as a graphic representation of the rate of oxygen consumption. In each experiment two basal metabolism determinations were made just prior to the application of the current. The animal was covered throughout the experiment with thick cotton insulating material and a rubber sheet. Blood was taken at intervals from a peripheral vein, usually in the hind leg. The samples for gas determination were stored under paraffin oil. Carbon dioxide content estimations were made with the aid of a Van Slyke portable manometric blood gas apparatus. Blood sugar was determined by Benedict's method, nonprotein nitrogen by Folin's method, and chlorides by Van Slyke's method.

The current used had the following characteristics: 1,000,000 cycles,

relatively high voltage, 500–3300 milliamperes.[1] Block tin electrodes (9 × 11 cm.) were placed on either side of the head or on the left upper arm and right thigh. Temperature measurements were made with copper-constantan thermocouples, inserted in hypodermic needles, and special mercury and spirit thermometers.[2] The thermocouples were calibrated through the temperature range used before each experiment. These thermocouples were especially designed for this work and will be described in detail elsewhere. Radio frequency chokes in the galvanometer leads were used to enable us to read the temperatures while the high frequency current was on without injuring the galvanometer or causing errors in the readings.

RESULTS ON ENERGY METABOLISM. Table 1 contains a few representative metabolism data. The times given in the second column indicate the termination of the metabolism periods which were of 20 to 30 minutes' duration. The average body temperature includes, in each case, the rectal and one to three temperatures in other regions of the body not in a straight line between the electrodes. Where the temperatures were not determined at the time given, they were obtained graphically from the temperature curve. Since these curves are rather smooth, this does not lead to any great error. A number of the respiratory quotients are very high but this is to be expected with such sudden increases in the respiratory rate.

In the first three experiments described, bitemporal electrodes were used. In experiment 1 a rise of 7.3°C. in body temperature was effected in 72 minutes. The heat production was increased 55 per cent and the rate of respiration 22-fold. The temperature rise in experiment 2 was only 3.0°C. in 81 minutes with an accompanying rise in metabolism of 27 per cent. The dog used in experiment 3 (table 1) showed a rise in average body temperature of 6.6°C. in 3¼ hours (i.e., to 12:34). With minor irregularities, the metabolism curve paralleled the temperature curve up to the maximum for the latter, the increase in metabolism being 50 per cent at this point. However, when the current was shut off (i.e., at 1:24) the metabolism dropped to basal in 3¼ hours whereas the temperature was lowered only 0.8°, *i.e.*, to 40.1°. The respiratory rate fell to less than one-third of the value at the maximum temperature. In the subsequent experiments the electrodes were shifted from the head to the left upper arm and the right thigh. In experiment 4, the highest body temperature was attained in the last metabolism period, a rise of 7.9° in 2½ hours. The maximum metabolism, also reached in this period, was 107 per cent above

[1] We are very glad to acknowledge the loan of high frequency equipment by Mr. Steinkamp of the Picker Co. and Mr. Renaud of the Victor X-Ray Corporation.

[2] We wish to thank Dr. Noyes of the Taylor Instrument Co. for supplying special thermometers.

TABLE 1

Metabolism of anesthetized dogs before, during and after diathermy.
(Typical experiments)

(Bitemporal electrodes)

EXPERIMENT NUMBER AND DATE	METABOLISM PERIOD	TIME END OF PERIOD	CALORIES PER HOUR	OBSERVED R.Q.	RESPIRATORY RATE PER MINUTE	AVERAGE BODY TEMPERATURE
						°C.
3. (2–3–30)	1	8:50	38.2	0.83	7	....
Weight 27.1 kgm.	2	9:20	? 44.0	? 0.65	7	34.3
Current on	3	10:11	40.5	0.85	9	35.7
Current on	4	10:37	47.2	0.91	13	37.1
Current on	5	11:03	46.3	0.83	11	39.1
Current on	6	11:40	50.3	0.85	21	39.7
Current on	7	12:04	50.4	0.92	68	40.2
Current on	8	12:34	57.5	0.83	102	40.9
Current on	9	12:58	57.0	0.79	90	40.7
Current off	10	1:24	55.5	0.89	60	40.8
Current off	11	3:18	37.8	0.89	30	40.1
(Arm and leg electrodes)						
5. (2–17–30)	1	10:44	29.7	0.96	12	....
Weight 18.6 kgm.	2	11:09	32.4	1.23	15	34.0
Current on	3	11:55	40.2	0.96	72	35.8
Current on	4	12:20	56.6	1.00	145	36.4
Current off	5	12:48	60.4	0.94	>250	37.3
Current off	6	1:17	70.6	0.84	>250	38.2
Current off	7	2:16	47.0	0.83	>250	39.1
Current on	8	2:41	52.5	1.07	>250	41.0
Current on	9	3:06	73.5	0.90	200	41.9
Current on	10	3:32	70.7	0.76	200	42.1
6. (3–3–30)	1	11:53	28.9	1.03	10	35.7
Weight 21.7 kgm.	2	12:17	30.0	1.00	12	35.1
Current on	3	12:55	39.4	1.15	19	37.0
Current on	4	1:22	42.1	0.95	17	38.8
Current on	5	1:47	59.7	0.83	>250	40.1
Current on	6	2:11	63.5	0.98	>250	40.9
Current on	7	2:43	75.7	0.83	>250	42.3
Current off	8	3:07	73.8	0.92	>250	42.3
Current off	9	3:30	66.6	0.85	135	43.4
Current off	10	3:50	49.0	0.80	85	41.6
Controls						
9. (7–24–30)	1	10:57	12.7	0.94	8	34.7
Weight 9.8 kgm.	2	12:13	11.7	1.00	5	34.1
	3	1:20	11.5	0.93	5	34.0
	4	2:50	11.6	0.87	6	34.4
10. (9–5–30)	1	11.47	26.7	0.96	12	35.5
Weight 22.9 kgm.	2	12:47	26.0	0.84	11	35.0
	3	1:47	27.2	0.86	12	34.3
	4	2:47	25.4	0.78	9	33.2

the average basal. In spite of a rising temperature in the next to the last period, the metabolic rate was appreciably lower than in the preceding one. Since the rate of ventilation steadily increased, the change in energy output cannot be charged to diminished respiratory effort. In experiment 5 (table 1) the current was shut off at the end of the fourth metabolism period and not turned on again until the early part of the eighth period. Nevertheless, the body temperature continued to rise and the metabolic rate increased up to the sixth period at which point it declined sharply from 70.6 to 47.0 Cals. per hour. With reapplication of the current the

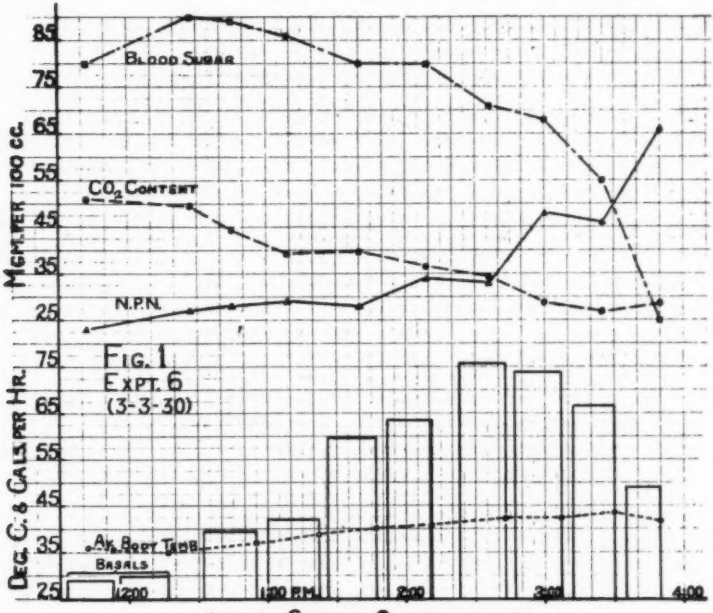

Fig. 1

energy output steadily rose to its previous maximum of 127 per cent above the basal level. During most of the time that the current was shut off, the animal was breathing very rapidly and at times was panting. It was rather difficult to count the actual number of respirations recorded because they were too close together on the record. However, it is plain that the accelerated respiratory rate, which continued after the current was cut off, was insufficient to account for the large rise noted in the level of the metabolism. In experiment 6 (table 1) the total observed rise was from 35.1° to 43.4°—a rise of 8.3° in $3\frac{1}{4}$ hours. The maximum metabolism,

157 per cent, was observed in the seventh period, about ¾ hour before the peak of the temperature curve was attained. Here again the energy output declined rapidly when the current ceased to flow and this occurred in spite of a body temperature which was maintained at a very high level (fig. 1). In this case the ventilation rate showed an appreciable diminution when the current was shut off. The dog used in experiment 7 had an average basal metabolism of 23.5 Cals. per hour. With the application of diathermy this was raised to 63.9 Cals. per hour—an increase of 172 per cent. The maximum rise in temperature was 7.8° which was recorded during the period of greatest energy output by the animal. The respiratory rate increased steadily. Experiment 8 showed the largest increase in body temperature—8.5° in 3 hours. In the first three periods of diathermy the intensity of the metabolism increased steadily but in the fourth and fifth there was a decided decline until in the next to the last period the metabolism at 42.2° was not a great deal in excess of the metabolism at 38.1°. The maximum energy output was 99 per cent above basal. There was the usual steady increase in respiratory rate.

Experiments 9 and 10 were controls which were continued for about 4 hours. The metabolism in experiment 9 was diminished by about 10 per cent, while the temperature was depressed less than 1.0°. In experiment 10 the heat production showed very small fluctuations, the tendency being toward a diminution. The body temperature was reduced more than 2.0°. There was a definite rise in non-protein nitrogen (table 2) but it did not approach the values found following treatment with diathermy. The blood chloride concentration decreased perceptibly.

Chemical results. Some of the blood chemistry findings are arranged in table 2. In each of the experiments 3, 5, 6, and 7, a fairly good series of analyses was made. In general they all show the same type of result, namely, 1, a rather rapid diminution in CO_2 content; 2, hypoglycemia; 3, greatly increased non-protein nitrogen values, and 4, no appreciable change in chlorides. The relatively constant chloride concentration is rather surprising in view of the hematocrit determinations made in experiments 7 and 8, which seem to show a considerable concentration of the blood. As the average body temperature approaches 41° the blood sugar concentration falls quite rapidly and simultaneously the non-protein nitrogen begins to rise above the normal level. The CO_2 content, on the other hand, is diminished rapidly but quite uniformly throughout the entire time that the diathermy is being applied. The blood chemistry findings in the controls are relatively constant.

Discussion. In this short series of experiments there is apparently no quantitative relation between the rise in temperature and the increase in respiratory metabolism. A small increase in body temperature is accompanied by a small increase in metabolism (27 per cent in expt. 2), but on

TABLE 2

Blood chemistry data of anesthetized dogs before, during and after diathermy

EXPERIMENT NUMBER AND DATE	TIME SAMPLE TAKEN	CO_2 CONTENT	SUGAR	N.P.N.	CHLORIDES	AVERAGE BODY TEMPERATURE
			mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*°C.*
3. (2–3–30)	9:45		60	30	515	34.3
Current on	10:27		70	31	490	36.5
Current on	11:00		80	26	525	38.3
Current on	11:35		—	35	540	39.6
Current on	12:00		60	37	480	40.2
Current on	12:27		60	40	480	40.9
Current on	12:57		55	43	465	40.7
Current off	1:23		50	65	515	40.7
Current off	3:05		50	73	—	—
Current off	3:13		40	92	515	—
5. (2–17–30)						
Current on	11:20	42.9	115	31	475	
Current on	11:57	48.1	105	31	465	
Current off	12:17	44.6	98	31	465	
Current off	12:37	41.0	80	33	455	
Current off	1:07	40.2	80	35	480	
Current on	2:13	43.6	87	35	440	39.1
Current on	2:40	37.7	85	40	440	41.0
Current on	3:06	33.5	75	43	420	41.9
Current off	3:33	—	65	48	420	42.1
	3:50*	35.6	40	63	440	43.2
6. (3–3–30)	11:42	50.9	80	23	540	36.0
	12:27	49.4	90	27	515	35.3
Current on	12:45	44.3	89	28	525	36.3
Current on	1:09	39.3	86	29	520	38.1
Current on	1:40	39.8	80	28	540	40.1
Current on	2:09	36.6	80	34	500	40.9
Current on	2:36	34.3	71	33	500	41.7
Current off	3:00	28.6	68	48	500	42.5
Current off	3:25	26.8	55	46	480	43.1
Current off	3:50*	28.5	25	66	515	41.6
			Controls			
9. (7–24–30)	11:27	—	82	27	500	34.2
	12:08	61.9	78	26	490	34.1
	1:30	62.2	90	24	500	34.0
	3:30	62.8	88	26	500	34.4
10. (9–5–30)	11:55	58.7	96	35	415	34.8
	1:00	54.5	100	36	383	34.9
	2:00	54.0	97	39	367	34.2
	3:00	52.4	94	43	354	32.8

* Blood from heart.

the other hand, the greatest increase in body temperature succeeded only in doubling the metabolic rate. Intermediate increments in temperature rise succeeded in elevating the metabolism 172 per cent (expt. 7). This does not fit in very well with the idea of a temperature coefficient for metabolic processes in this type of experiment. The Q_{10} for these experiments was calculated but it exhibited such wide variability and such high values that it was not included in this report. Although the rate of temperature increase is probably a factor, it does not seem to bear any simple relation to the change in energy output of the animals used in these experiments. The work of Landis and co-workers (1926) shows that a rapid elevation of body temperature in a hot water bath is accompanied by an oxygen consumption considerably in excess of the amount to be expected on the basis of the temperature coefficient of DuBois (1927) for fever patients. This phenomenon also appears in the work of McConnell and Yagloglou (1925) who studied the effects of hot air on metabolism. Bazett (1927) suggested that the extra work entailed in the increased respiration might account for a large portion of the oxygen consumed, especially if the animal were immersed and forced to displace water with each inspiration. He also called attention to the fact that mean body temperature cannot be determined by any single measurement such as the oral or rectal temperature. These suggestions are not entirely applicable to our experiments since our dogs were not immersed. Furthermore, the body temperatures which we report are averages obtained from several simultaneous determinations made in different regions of the body. All that can be definitely stated from a consideration of our data is that a rise in average body temperature of about 8°C. was accompanied by an increase in respiratory metabolism of 100 per cent or more.

As pointed out in the presentation of results, there are several instances in which the metabolism decreased when the current was shut off even though the average body temperature continued to rise or remained constant (fig. 1). As yet we have no explanation for these results. Before they can be explained more data must be obtained on the work performed during respiration and the possible direct effect of high frequency current upon the oxygen consumption of tissues as well as upon the whole animal.

The great lowering of blood and plasma CO_2 content is to be expected with the extreme hyperventilation noted in most of these experiments. The lowered CO_2 tension in the tissues which must occur is, likely, a factor in determining the amount of O_2 which can be obtained from the circulating blood. Estimation of the actual respiratory quotients is impossible under these conditions. Certain corrections can be made by calculating the amount of CO_2 lost from the blood stream from one blood sample to the next but this involves an assumption with regard to the total blood volume and the correction, therefore, has not been made. In making the

heat calculation the calorific value of oxygen at the observed R.Q. has been used. The value 5.047 Cals. per liter was used for all R.Q.s above unity. This method, obviously, leaves much to be desired in the way of accuracy but at present we are unable to offer anything better. In consequence of this the fuel of the increased metabolism cannot be determined by the R. Q. The hypoglycemia found in the longer experiments indicates that the carbohydrate stores are heavily drawn upon.

Whether or not urea constitutes the greater portion of the high concentration of blood non-protein nitrogen, we are unable to say. If this is found to be true, it will indicate an appreciable destruction of body protein at about 41–42°C. It is probable, however, that the source of this non-protein nitrogen may be localized in the region of the electrodes where the cutaneous and subcutaneous temperatures were high enough to cause edema and coagulation of some of the tissues. The histological changes will be discussed in a later paper.

If the absolute quantity of chlorides suffered any gross changes, they were obscured by factors as yet unknown to us.

SUMMARY AND CONCLUSIONS

Experiments have been described which show that high frequency current as applied to anesthetized dogs is capable of raising their average body temperature 3.6 to 8.5°C. The respiratory metabolism as determined by the Benedict Universal apparatus was increased 27 to 172 per cent. The rate of respiration in some cases increased twenty-five fold. Whole blood CO_2 content, as well as plasma CO_2 content, was decidedly diminished. When the average body temperature reached 41–42°C. the blood sugar concentration fell rapidly while the non-protein nitrogen concentration showed a large increase. The blood chloride concentration failed to exhibit any gross change. The location of the electrodes whether across the head or across the trunk did not influence the character of the results.

We wish to express our gratitude to Prof. John R. Murlin for his aid and counsel during the course of this investigation. We wish also to thank Miss Dorothy Jamison for her coöperation in the blood chemistry studies.

BIBLIOGRAPHY

D'Arsonval, A. 1893. Arch. de physiol., Series 5, v, 401.

Bazett, H. C. 1927. Physiol., Rev., vii, 531.

Bettman, R. B. and N. N. Crohn. 1927. Journ. Amer. Med. Assoc., lxxxviii, 532.

Binger, C. A. L. and R. V. Christie. 1927. Journ. Exper. Med., xlvi, 571, 585, 595.

Bischoff, F., H. J. Ullmann, E. Hill and L. M. Long. 1930. Journ. Biol. Chem., lxxxv, 675.

Christie, R. V. and C. A. L. Binger. 1927. Journ. Exper. Med., xlvi, 715.

DuBois, E. F. 1927. Basal metabolism in health and disease. Philadelphia.
Durig, A. and A. Grau. 1913. Biochem. Zeitschr., lxviii, 480.
Landis, E. M., W. L. Long, J. W. Dunn, C. L. Jackson and U. Meyer. 1926. This Journal, lxxvi, 35.
Lonergan, R. C. 1927. Journ. Indust. Hyg., ix, 1.
McConnell, W. J. and C. P. Yagloglou. 1925. Arch. Int. Med., xxxvi, 382.
Nagelschmidt, F. 1926. Lehrbuch der Diathermie. Berlin.
Nasset, E. S. and S. L. Warren. 1930. Proc. Soc. Exper. Biol. Med., xxvii, 943.
Stary, Z. 1926. Zeitschr. f. Biol., lxxxv, 138.

I. BILE PIGMENT AND HEMOGLOBIN INTERRELATION IN NORMAL DOGS

KHUN SRIBHISHAJ,[1] WILLIAM B. HAWKINS AND GEORGE H. WHIPPLE

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York

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In spite of the very considerable amount of experimental work related to bile pigment elimination by the body we believe there is much needed information not yet available. We submit evidence below which does not square with some present day conceptions relating to pigment metabolism. There has been a tendency to look upon the pyrrol nucleus as the essential determining factor in bile pigment production and new hemoglobin formation within the body. If this is true, then we believe the evidence shows that under certain conditions the body can synthesize the pyrrol nucleus readily on demand in considerable amounts. If it be accepted that the body can assemble the pyrrol nucleus in an emergency it must be admitted that this reaction may go on under ordinary conditions.

Hooper, Smyth, Smith and Whipple at various periods have had considerable experience with various types of bile fistula in dogs. Each type of fistula has good and bad features but one fistula may be well suited to one type of investigation and not to another. The familiar open fistula was used by Hooper and Whipple (1) in their experiments to study bile pigment metabolism. This type of open bile fistula is associated with infection of the bile passages which at times of sluggish bile flow may permit of a reduction of bile pigment to urobilin within the biliary tree. This of course introduces a serious error which is hard to control.

By far the best type of fistula for bile pigment study is the renal type described under "methods." In this fistula the bile flows into the normal renal pelvis and is readily collected with the urine in a standard metabolism cage. There is no infection of the biliary passages, renal pelvis, ureter or urinary bladder and the dogs remain in perfect condition indefinitely if they are fed some fresh dog bile by mouth daily.

For the sake of comparison and control we give some experiments with a closed sterile bile fistula as described by Rous and McMaster (2). The findings are very similar when compared with the renal type of fistula. The closed sterile fistula has disadvantages for bile pigment work aside

[1] Rockefeller Foundation Visiting Fellow.

from the time-consuming care of the dogs to insure sterile bile. The tube may be kinked and cause partial or complete obstruction; also salt, mucus or other substances within the tube may adhere to the wall and gradually shut off the bile flow. It is unusual for a closed sterile fistula to remain in good clinical condition with regular bile flow for more than 3 to 4 months. We have a renal bile fistula under observation more than a year since the operation and the dog (26–45) is in perfect condition. Another dog (29–34) is in perfect condition after seven months' observation. A third dog lived over 3 years even with a severe experimental anemia added to the renal bile fistula (dog 26–29, table 22).

That bile feeding has no direct influence on the output of bile pigment in either the renal or closed fistula or in the normal dog has been amply proven by experiments in this laboratory, by Blankenhorn (3) and by Bollman, Sheard and Mann (4). Bile pigment is reduced in the intestine to urobilin and some urobilin is absorbed as has been established by McMaster and Elman (5), but evidently this urobilin has no relation to the output of bile pigments. All evidence points to bilirubin as an end product which cannot be rescued from the intestine to produce new bilirubin or hemoglobin.

METHODS. In these bile pigment experiments the *gall bladder renal fistula* devised by Kapsinow, Engle and Harvey (6) has proved to be highly satisfactory for this work. The operation can be performed in one stage, and the technique is not difficult, but we have met occasionally with post-operative complications such as blood clot in renal pelvis causing obstruction to the fistula, or hemorrhage from the incised kidney. In one instance the fistula opening was too small, and the bile did not flow freely. For purposes of comparison we have used the sterile closed fistula devised by Rous and McMaster and modified by Smith, Groth and Whipple (7).

The renal fistula dogs are kept in galvanized iron metabolism cages, and the urine bile mixture is collected over 24-hour periods. As a preservative 5 cc. chloroform are put in the collection bottle. The dogs are given 50 cc. of dog's bile and 250 cc. of water daily by stomach tube three hours before the collection of the 24-hour samples. The water is given as it causes the dogs to void regularly before the collections of the urine, and the daily amounts of bile pigments are more uniform as a result. Catheters are never used because a cystitis destroys the usefulness of a dog.

The *quantity of bile pigment* in the urine is determined as follows: 10 and 20 cc. portions of the measured 24-hour samples are made alkaline with ammonia and then 10 cc. of 10 per cent calcium chloride are added which causes a voluminous precipitate to form. The precipitate is thrown down by use of the centrifuge, the supernatant clear fluid is decanted and the precipitate dissolved in 10 cc. 95 per cent alcohol to which 2 cc. of concentrated hydrochloric acid are added. The solution is poured into a

50 cc. volumetric flask, 0.5 to 1.0 cc. concentrated nitric acid added and then alcohol up to the mark. The blue green color develops in a few minutes and the pigments are estimated by the colorimeter. The development of the color may be hastened if necessary by placing the flask in hot water.

In determining the amount of pigment in the bile from the closed fistula dogs 1 cc. of the bile is added to 49 cc. of an acid alcohol solution in a volumetric flask, mixed and allowed to stand until the bluish green color develops. The pigment is estimated colorimetrically. The acid alcohol solution consists of 95 per cent ethyl alcohol 100 cc., nitric acid concentrated 0.4 cc., hydrochloric acid concentrated 2 cc. If the nitric acid used contains a slight trace of nitrous oxide the pigment color will develop much more rapidly.

The *standard for bile pigment* is made up of 0.3 cc. of a one per cent solution of potassium bichromate plus 40 cc. of a 10 per cent solution of copper sulphate. This standard solution was described by Rous and McMaster (8) and in this laboratory is checked against samples of pure bilirubin. From this standard solution colorimetric readings enable the investigator readily to estimate milligrams of bile pigment in any solution. A Wratten filter no. 72 is used in reading these colors.

Repeated controls of this method are made from time to time by adding bile containing a measured amount of bile pigment to the renal fistula urine or to normal urine. Experience shows that in our hands about 90 to 95 per cent of the bile pigment added to urine can be recovered. This is a constant figure and represents a loss due to method of analysis.[2] This indicates that our figures given below in renal fistulas are 5 to 10 per cent too low.

The *standard basal ration* is a bread made in the laboratory and described elsewhere (9). It is in itself a complete ration which will maintain normal dogs in health and normal activity indefinitely. It contains wheat flour, bran, potato starch, canned salmon, sugar, cod liver oil, tomatoes, yeast and a salt mixture. This diet permits a minimum regeneration of new hemoglobin in anemia and because its pigment producing properties were in part understood we felt it was especially suited to bile fistula work. This bread is well liked by the dogs but at times it may be supplemented by small amounts of canned salmon or "Klim."

In preparing hemoglobin for injection the blood is centrifugalized for about 30 minutes, the plasma sucked off and then the packed cells are washed twice with physiological saline. Two volumes of distilled water are added to the washed packed red cells, which are then laked, centrifugal-

[2] In working out and controlling the method for accurate analysis of bile pigment in urine, Dr. A. L. Bassin and Dr. D. S. Martin did much preliminary work which was an essential part of this problem.

ized and the hemoglobin solution filtered through gauze. One cubic centimeter of the hemoglobin solution is diluted with 99 cc. 0.1N hydrochloric acid and allowed to stand 3 hours, after which the hemoglobin value is estimated using a one per cent solution of acid hematin as standard (10). This solution of hemoglobin is injected into the jugular vein in amounts equivalent to 2 grams hemoglobin as this amount is known to be below the hemoglobin threshold of these dogs (11). The blood hemoglobin level is determined following the method of Robscheit (10).

AUTOPSY FINDINGS. The usual findings at autopsy on the *closed fistula* dogs are as follows: the rubber tubing is encased in a fibrous sheath which shows foreign body giant cells but no infection. The common and hepatic ducts are slightly dilated, but free from infection. The liver grossly appears normal. Microscopically one finds usually some slight round cell infiltration of the portal spaces but the small ducts are normal. The other organs present nothing of especial interest.

In *renal fistula* dogs, the gall-bladder fundus is found nicely healed to the kidney pelvis. The common bile duct is examined with especial care to be certain that the duct is completely separated from the duodenum. The ureter is patent with smooth mucosa, the renal pelvis and gall bladder show a normal mucosa free from infection. The bile ducts show slight if any dilatation. The portal spaces of the liver show no round cell infiltration and the liver appears essentially normal. The right kidney shows some scar tissue at the site of the operation, as would be expected. Other organs present no significant abnormalities.

EXPERIMENTAL OBSERVATIONS. In these experiments we wish to stress the excellent clinical condition of these dogs with the renal-bile fistula and we believe these experiments approximate more closely the theoretical normal than any as yet published. All too frequently in bile fistula experiments one finds no note as to the clinical condition of the dogs but a weight curve showing a loss of 3 to 4 kilos during a few weeks. One familiar with this type of experimental animal at once suspects an infection of the biliary tree with intoxication. Gastro-intestinal upsets may be observed and cause loss of weight and modifications of bile secretion. Duodenal ulcers may develop on a bile and liver free diet and cause anorexia and loss of weight.

These bile fistula dogs were in weight equilibrium or even gained weight, ate an abundant standard ration, were clinically normal and vigorous and presented normal blood hemoglobin levels. They were confined to clean metabolism cages at all times so that the amount of exercise was limited. Under these conditions one feels confidence in the daily output level of bile pigment and departures from this mean have real significance.

Table 1 is of especial interest because of the long period of observation available—compare the next paper, tables 23 to 25, which together give

the bile pigment history of this dog over a period exceeding 12 months. During all this time the dog was in excellent clinical condition and in all respects normal but for the experimental anemia induced subsequent to table 1. This dog is still under observation and is in perfect clinical condition. The control period is made up of the fore and after control days which together are equal in number to the days of hemoglobin injection.

TABLE 1

Bile pigment recovery from hemoglobin injection

Dog 26–45, normal renal fistula, operation May 1, 1929

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	URINE BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
July 24	55	0	139	700	
July 25	44	0		600	
July 26	63	0		620	12.9
July 27	63	0		570	
July 28	56	0		450	
July 29	54	2		550	
July 30	124	2		425	
July 31	124	2		350	
August 1	118	2		550	
August 2	136	2		435	12.2
August 3	124	2		400	
August 4	120	2		525	
August 5	138	2		700	
August 6	132	2		550	
August 7	130	2	140	720	
August 8	124	2		700	12.9
August 9	119	2		820	
August 10	129	0		680	
August 11	48	0		580	
August 12	56	0		910	
August 13	53	0		750	
August 14	65	0		500	
August 15	67	0		710	
August 16	59	0	132	400	12.6

Average bile pigment output for 24 hours during both control periods = 57 mgm. or 4.4 mgm. per kilo.

For this control period the bile pigment output per 24 hours is 57 mgm. or 4.4 mgm. per kilo. Each day's hemoglobin injection is reflected in the bile collection of the following day as the hemoglobin solution is given in the afternoon following the daily bile-urine collection.

During this observation this dog was fed daily the following ration—salmon bread 300 grams, canned salmon 100 grams, Klim 25 grams, made

into a mash with water. This was consumed well and there was no weight fluctuation. The dog received daily 75 cc. dog bile by stomach tube as described under "methods."

The total control bile pigment output is 683 mgm. and the total bile pigment output during hemoglobin injections is 1518 mgm. The excess due to hemoglobin injection is therefore 835. Theoretically 24 grams hemoglobin are equivalent to 960 mgm. bile pigment if there should be a quantitative elimination. This gives a recovery ratio of 835 over 960 or 87 per cent as given in table 7. If we allow for a 10 per cent loss due to

TABLE 2

Bile pigment recovery from hemoglobin injection

Dog 29–34, normal renal fistula, operation September 27, 1929

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	URINE BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
March 11	122	0		660	17.5
March 12	133	0		900	
March 13	135	0		940	
March 14	134	2		1,125	
March 15	185	2	119	860	
March 16	201	2		570	
March 17	214	2		925	17.7
March 18	185	2		720	
March 19	199	2		745	
March 20	137	2		500	17.9
March 21	169	2		1,230	
March 22	194	0		845	
March 23	139	0		860	
March 24	116	0		675	17.9
March 25	117	0		865	
March 26	101	0		575	

Average bile pigment output for 24 hours during both control periods = 124 mgm. or 7 mgm. per kilo.

the method of bile pigment analysis of the urine bile mixture we come very close to a theoretical 100 per cent return of the pyrrol nucleus split off from the hemoglobin molecule to appear as bile pigment surplus.

Table 2 shows another valuable bile fistula which has been under observation more than six months. The dog has remained in excellent clinical condition and except for an experimental anemia, normal in all respects. The dog is under observation at present and shows no change in body weight or activity. This dog during this observation period (table 2) received and ate well the following daily ration—salmon bread 350 grams,

canned salmon 100 grams, Klim 50 grams, made into a mash with water. He was given daily by stomach tube 70 cc. dog bile and 250 cc. water. The bile pigment levels therefore have an especial interest during the control periods as this dog shows a high bile pigment output level—the highest by a good deal in our entire colony.

This dog (table 2) produces during control periods 124 mgm. bile pigment per 24 hours, or 7 mgm. per kilo. This dog continues to show higher levels than other dogs during anemia periods (paper II) and we may say that his pigment forming machine is geared for higher speed than the average. We see no reason to assume that this dog destroys red cells more rapidly than normal but he possesses this personal idiosyncrasy just as some dogs are more speedy and have larger hearts than others or produce more hemoglobin on standard diets in anemia than other dogs.

This dog (table 2) shows a total bile pigment production during control periods of 997 mgm. and an output of 1484 mgm. bile pigment during the hemoglobin injection periods. This pigment surplus is 487 mgm. resulting from 16 grams hemoglobin injection, or a recovery ratio of 487 over 16 $\times$ 40 or 76 per cent—consult table 7. This percentage recovery is somewhat below the 87 per cent recorded in table 1.

In spite of an unusually high average daily bile pigment output this dog puts out less bile pigment per gram of introduced hemoglobin than does the dog in table 1. One might have expected to find exactly the contrary, reasoning from theoretical considerations alone.

We regret that more blood hemoglobin figures are not available during this experimental period (table 2) but from observations before and after this period and from the weight curve and general clinical condition we feel confident that there was no significant fluctuation in circulating hemoglobin during this observation period or later.

Table 3 and table 4 should be examined together as the same dog is observed during consecutive months. The fistula operation was performed on September 9 and following the operation there were no post-operative difficulties. The dog before operation weighed 12.4 kgm. and was a common mongrel in a rather poor state of nutrition. As will be seen from tables 3 and 4, he gained steadily in weight following the operation and remained in excellent clinical condition. This dog was given a daily feeding consisting of salmon bread 400 grams, canned salmon 100 grams, Klim 50 grams, mixed as a mash with water. This was well eaten. He was also given 50 cc. dog bile plus 300 cc. water by stomach tube as explained under "methods."

This régime causes a progressive gain in weight to a maximum (table 4) of 17.9 kgm. In spite of this gain of weight we note no change in the average daily control bile pigment excretion. We wish to emphasize that this dog shows a control level for bile pigment output per 24 hours of 80

mgm. in the lean period (table 3) and 82 mgm. bile pigment per 24 hours after the weight had returned to normal (table 4). Figured on the basis of 14 kgm. (table 3) we would record 5.8 mgm. per kilo but later after a gain of 3 kgm. we figure 4.8 mgm. per kilo (table 4). It seems to us obvious that the *total bile pigment output for a given dog is relatively constant even during wide fluctuations in body weight.* Individual dogs may differ widely in this average bile pigment output but this is a personal idiosyncrasy which we cannot explain at present.

This dog during the first period shows a control output (table 3) of

TABLE 3

Bile pigment recovery from hemoglobin injection

Dog 29–6, normal renal fistula, operation September 9, 1929

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	URINE BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
October 17					13.0
October 19	106	0		1,020	
October 20	51	0		805	
October 21	65	0		1,155	13.5
October 22	69	2.5	116	870	13.8
October 23	142	2.5		800	
October 24	204	2.5		755	13.6
October 25	167	2.5		685	
October 26	205	2.5		865	
October 27	157	2.5		750	
October 28	164	2.5		1,155	
October 29	123	0		725	14.8
October 30	85	0	117	815	14.7
October 31	89	0		975	
November 1	97	0		945	

Average bile pigment output for 24 hours during both control periods = 80 mgm. or 5.8 mgm. per kilo.

562 mgm. bile pigment and during the hemoglobin injection a bile pigment output of 1162 which gives a surplus of 600 mgm. bile pigment due to the hemoglobin injection. This gives a recovery ratio of 600 over 17.5 × 40 or 86 per cent return from the injected hemoglobin—refer to table 7.

The same dog later (table 4) shows a control output of 827, a hemoglobin injection output of 1627 and a surplus due to hemoglobin injection of 800 mgm. bile pigment. This gives a recovery ratio of 800 over 20 × 40 or 100 per cent return.

This dog (table 4) some time after these observations were recorded was used for an experiment in which digested beef hemoglobin was given intra-

venously. This caused shock and death after some hours. The *autopsy* findings are of no particular interest except to show that the renal bile fistula tract was in perfect condition.

Tables 5 and 6 are to be compared as the same dog is under observation. This dog presents the *closed sterile bile fistula* as described under "methods." This gives a control for the renal bile fistula and shows that the average control bile pigment output is about the same in both types of fistula.

TABLE 4

Bile pigment recovery from hemoglobin injection

Dog 29–6, normal renal fistula

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	URINE BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
November 16	85	0		955	16.6
November 17	81	0		915	
November 18	77	0		1,160	16.9
November 19	89	0		1,000	
November 20	72	2	135	900	
November 21	153	2		950	
November 22	157	2		850	16.4
November 23	201	2		1,090	
November 24	148	2		975	
November 25	182	2		1,135	17.2
November 26	168	2		990	
November 27	138	2		1,025	
November 28	166	2		980	
November 29	178	2		1,240	16.8
November 30	136	0	121	1,420	
December 1	95	0		780	
December 2	84	0		1,660	17.9
December 3	76	0		1,060	
December 4	77	0		650	
December 5	91	0		750	

Average bile pigment output for 24 hours during both control periods = 82 mgm. or 4.8 mgm. per kilo.

This dog 28–298 was in good clinical condition and shows slight weight fluctuations with a weight level a little higher during the last experiment (table 6). Three experiments with hemoglobin injection are recorded for this dog, the first in table 5, the third in table 6 and the second in abstract form in table 7.

This dog (28–298) was given a daily diet made up as follows—salmon bread 400 grams, canned salmon 100 grams and Klim 50 grams, made into

a mash with water. No bile was given by mouth during this observation period.

Table 5 shows a typical experiment exactly like those recorded with the renal bile fistula but this dog presents a *sterile closed* fistula. In this experiment we note a bile pigment output per 24 hours of 93 mgm. during control periods. The series of hemoglobin injections gives a total bile

TABLE 5

Bile pigment recovery from hemoglobin injection

Dog 28–298, normal sterile closed fistula, operation July 13, 1929

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
August 9	61	0		80	15.4
August 10	100	0		137	
August 11	91	0		112	
August 12	78	0		162	
August 13	60	0		170	
August 14	59	2	147	124	
August 15	121	2		132	
August 16	148	2		143	
August 17	166	2		126	
August 18	165	2		157	15.7
August 19	174	2		134	
August 20	173	2		111	
August 21	86	2		108	
August 22	87	2		135	
August 23	100	2		95	
August 24	174	2		112	
August 25	157	0		162	
August 26	119	0		159	
August 27	124	0		115	
August 28	70	0		91	15.5
August 29	163	0		154	
August 30	93	0		130	

Average bile pigment output for 24 hours during both control periods = 93 mgm. or 6 mgm. per kilo.

pigment output of 1551 mgm. bile pigment and the total control output is 1018 mgm. giving a bile pigment surplus of 533 mgm. due to the hemoglobin injection. This gives a recovery ratio of 533 over 22 × 40 or 61 per cent. This is a low recovery but subsequent experiments give higher values of 80 and 90 per cent respectively.

In table 5 we note from day to day considerable fluctuation in total bile output and bile pigment but at present we are unable to explain these

changes. There was no clinical disturbance, no change in weight and no bile infection.

Table 6 in many respects is more satisfactory than table 5, both observations being on the same dog. This period is 3 months later and there is no change in the clinical condition and weight is unchanged. The diet intake is the same and no bile is given by mouth.

The output of bile pigment is more uniform in both control and hemoglo-

TABLE 6

Bile pigment recovery from hemoglobin injection

Dog 28–298, normal sterile closed fistula

DATE	BILIRUBIN OUTPUT 24 HRS.	INJECTION HEMOGLOBIN 24 HRS.	BLOOD HEMOGLOBIN LEVEL	BILE COLLECTION 24 HRS.	WEIGHT
	mgm.	*grams*	*per cent*	*cc.*	*kgm.*
November 16	82	0		143	
November 17	71	0		146	
November 18	50	0		93	16.8
November 19	76	0		175	
November 20	82	2	131	147	
November 21	133	2		65	
November 22	171	2		100	15.8
November 23	146	2		114	
November 24	148	2		114	
November 25	131	2		120	16.4
November 26	157	2		121	
November 27	149	2		124	
November 28	183	2		110	15.9
November 29	188	2		87	
November 30	190	0	135	98	
December 1	121	0		93	
December 2	124	0		128	15.9
December 3	102	0		140	
December 4	88	0		172	
December 5	81	0		72	16.1

Average bile pigment output for 24 hours during both control periods = 88 mgm. or 5.5 mgm. per kilo.

bin injection periods but we note practically the same output average during control periods or 88 mgm. bile pigment per 24 hours. The recovery ratio after hemoglobin injections is much higher than recorded in table 5 and in this experiment amounts to 90 per cent.

Table 7 is given to summarize the results of the seven experiments, four with renal bile fistulas and three with closed sterile bile fistulas. The *recovery ratio* of bile pigment coming from the injected hemoglobin is of especial interest. Theoretically 1 gram hemoglobin if split down into

bilirubin and globin should yield 40 mgm. bilirubin. Therefore 10 grams hemoglobin given intravenously should yield 400 mgm. bile pigment if there is a quantitative shift of the pigment radicle of hemoglobin into bilirubin and complete elimination in the bile. We note in the renal bile fistula under the controlled conditions a recovery ratio of 87 per cent, the average of the four experiments. If we accept a loss of about 10 per cent due to method of analysis of bile pigment in dog urine, the actual recovery by the renal bile fistula would run about 95 per cent on the average or a close approximation to the theoretical return.

With the closed sterile fistula (table 7) the output of bile pigment averages in 3 experiments 77 per cent of the theoretical complete return from hemoglobin injection. The analysis of bile pigment in bile presents no difficulties and yet this figure is definitely lower than those just given for

SUMMARY TABLE 7

Hemoglobin injection and bile pigment recovery—normal dogs

DOG		TOTAL BILE PIGMENT OUTPUT			TOTAL HEMOGLOBIN INJECTED	BLOOD HEMOGLOBIN LEVEL	TYPE BILE FISTULA
Number	Weight	Control period	Hemoglobin injection period	Recovery ratio from hemoglobin			
	kgm.	*mgm.*	*mgm.*	*per cent*	*grams*	*per cent*	
29-6	14.0	562	1,162	86	17.5	117	Renal
29-6	17 0	827	1,627	100	20.0	128	Renal
26-45	12.5	683	1,518	87	24.0	136	Renal
29-34	17.7	997	1,484	76	16.0	119	Renal
28-298	15.5	1,018	1,551	61	22.0	147	Tube
28-298	15.5	662	1,229	81	17.5	126	Tube
28-298	16.2	877	1,596	90	20.0	135	Tube

the renal bile fistula. One might expect to get back more bile pigment from the closed sterile bile fistula because there is no possibility of loss due to analytical method difficulties. If we should discard the first observation on this dog (table 5) the remaining observations would come closer to the theoretical return but still fall below that of the renal bile fistula when allowance is made for loss of bile pigment in the analysis of the urine.

When hemoglobin is given intravenously there is always a slight loss by way of the urine as the bile pigment is formed in part in the blood stream and the bile pigment urinary threshold is so very low. This loss by way of the urine decreases the output from the closed fistula but obviously not with the renal bile fistula. The absolute values for such loss through the urine as applied to closed fistulas we cannot say but it is probably not large.

One may choose to believe that the renal bile fistula is more nearly normal and therefore returns a higher amount of bile pigment from a given

hemoglobin injection than does the other type of fistula. This assumes as normal a quantitative elimination of the pigment radicle of hemoglobin in the form of bilirubin when no anemia is present.

If however we admit that with *anemia* (paper II below) there will not follow a quantitative appearance of bile pigment coming from injected hemoglobin we must be prepared to accept various and sundry other emergencies in which material coming from hemoglobin or other substances may be diverted to other body uses rather than to follow the customary breakdown cycle to bilirubin. We hope to report further on these interesting possibilities.

Discussion. Stadelmann (12) many years ago using an open infected fistula found that dogs excreted 4.2 to 10.2 mgm. bilirubin per kilo per 24 hours. Hooper and Whipple (1) using the open infected bile fistula found the average in their series to be close to 9 mgm. bile pigment per 24 hours but they used 6 hour collections. McMaster, Brown and Rous (13) using the closed sterile fistula noted a range of 5.2 to 11 mgm. with an average of 7.4 mgm. bilirubin per kilo per 24 hours. Our values for bilirubin elimination with the renal-bile fistula fall usually within 4 to 6 mgm. per kilo per 24 hours.

These lower levels of bile pigment formation we believe are due in part to the excellent clinical condition of these dogs. They are under absolutely uniform conditions with a standard food intake, bile daily by mouth, uniform temperatures and restricted exercise. We believe these dogs approximate very closely the absolute normal for bilirubin production.

Does all this bilirubin excreted in these fistulas represent destroyed *blood hemoglobin*? This is the usual explanation but we believe there are several errors in this simple equation. *Muscle hemoglobin* when introduced into the blood stream promptly appears as bile pigment in the urine or fistula (14). It is safe to assume that muscle hemoglobin is worn out and replaced—therefore that an unknown volume of bile pigment from this source is contributed to the total bile pigment daily output. We hope to report further on this point.

We have noted the experiments of Seyderhelm, Tammann (15) and Baumann (16) who report a progressive anemia in a type of bile-urinary bladder fistula. This type of fistula is rather different in that a rubber tube connects the gall bladder and the urinary bladder. These dogs show a rather rapid loss of weight which in our experience indicates infection or gastrointestinal disturbances due to faulty diet. In our long experiments we have not noted a tendency to anemia when the renal bile fistula dog was in good clinical condition. They study the effect of various substances on this type of anemia and report that bile salts and irradiated ergosterol prevent the anemia. We hope to report experiments bearing on the various intoxications encountered in bile fistula dogs. We believe the

anemia noted by these workers is secondary to the intoxication resulting from this type of bile fistula and may be in part dietary.

SUMMARY

The renal type of bile fistula permits of bile pigment observation during long periods of time with the dog in perfect condition. In our hands this type of fistula gives bile pigment secretion figures which must approximate physiological normalcy.

The bile pigment output per 24 hours falls slightly below the figures recorded in the literature and averages between 4 and 6 mgm. per kilo.

The individual dog has its own total bile pigment output level which under uniform conditions shows a remarkably constant average value from month to month. This total output is not changed following gain or loss of weight but obviously the milligrams per kilo must change.

Dog hemoglobin solutions given intravenously under carefully controlled conditions show an increase in bile pigment elimination corresponding to 90 to 95 per cent of the expected increase if there were a quantitative transformation of hemoglobin to bile pigment. Theoretically 1 gram hemoglobin is equivalent to 40 mgm. bilirubin.

The closed sterile bile fistula is carried as control to the renal-bile fistula. This closed fistula shows a bile pigment increase due to hemoglobin injection which is less than the renal fistula and averages 75 to 80 per cent of the theoretical return.

These experiments serve as comprehensive control observations for the anemia experiments in the following paper.

BIBLIOGRAPHY

(1) Hooper and Whipple. This Journal, 1916, xl, 332.
(2) Rous, P. and P. O. McMaster. Journ. Exper. Med., 1923, xxxvii, 11.
(3) Blankenhorn. Journ. Exp. Med., 1927, xlv, 195.
(4) Bollman, Sheard and Mann. This Journal, 1926, lxxviii, 658.
(5) McMaster and Elman. Journ. Exp. Med., 1925, xli, 513.
(6) Kapsinow, R., L. P. Engle and S. C. Harvey. Surg., Gyn. and Obst., 1924, xxxix, 62.
(7) Smith, H. P., A. H. Groth and G. H. Whipple. Journ. Biol. Chem., 1928 lxxx, 659.
(8) Rous, P. and P. O. McMaster. Journ. Exp. Med., 1921, xxxiv, 47.
(9) Whipple, G. H. and F. S. Robscheit-Robbins. This Journal, 1925, lxxii, 395.
(10) Robscheit, F. S. Journ. Biol. Chem., 1920, xli, 209.
(11) Manwell, E. J. and G. H. Whipple. This Journal, 1929, lxxxviii, 420.
(12) Stadelmann. Der Icterus. Stuttgart, 1891.
(13) McMaster, Brown and Rous. Journ. Exp. Med., 1923, xxxvii, 395.
(14) Whipple, G. H. and F. S. Robscheit-Robbins. This Journal, 1926, lxxviii, 675.
(15) Seyderhelm and Tammann. Klin. Wochenschr., 1927, vi, 1177.
(16) Seyderhelm, Tammann and Baumann. Zeitschr. f. d. Ges. Exper. Med., 1929, lxvi, 539.

II. BILE PIGMENT AND HEMOGLOBIN INTERRELATION IN ANEMIC DOGS

W. B. HAWKINS, K. SRIBHISHAJ,[1] F. S. ROBSCHEIT-ROBBINS AND G. H. WHIPPLE

From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York

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Experiments were published by Whipple and Hooper (1) some time ago to indicate that a bile fistula dog would eliminate less bile pigment during anemic periods than during normal periods. This might be explained as due to conservation of pigment building material to form needed hemoglobin or to a decrease of hemoglobin split products coming from normal wear and tear of a smaller mass of circulating red cells.

Further experiments published by Whipple and Robscheit-Robbins (2) indicate clearly that dog hemoglobin introduced intravenously into a standardized anemic dog will result in new hemoglobin and red cell production in excess of the control periods amounting to 90 or 100 per cent of the hemoglobin introduced. This is evidence of a beautiful conservation of valuable material effected by the body in an emergency due to anemia. The evidence indicates that the introduced hemoglobin is broken down at least in part before it is reassembled to form the new hemoglobin within the red cells. We naturally assumed that the conserved portion consisted at least in part of the complex pyrrol nucleus. We approached the crucial experiments upon the anemic bile fistula with a definite conviction that we knew what was going to happen and could write the equation without the formality of a test by experiment.

From knowledge available it seemed pretty obvious that the following results were to be expected. Given a dog with severe anemia (about one-third normal blood hemoglobin level) and a satisfactory bile fistula one might introduce 30 grams of dog hemoglobin intravenously. One might expect thereafter to get from the bile an excess of bile pigment the equivalent of 10 to 15 grams of hemoglobin and from the blood the rest of the injected hemoglobin conserved to form new hemoglobin in red cells which could be removed by bleeding, amounting to 15 to 20 grams of hemoglobin. There being only 30 grams of hemoglobin introduced obviously one should expect no more than 100 per cent return—a part as bile pigment and the rest as new hemoglobin in red cells.

[1] Rockefeller Foundation Visiting Fellow.

The actual findings we refused to consider seriously until experiments had been done over and over again with every possible control. We believe now that it must be accepted that the reaction is totally different from what was anticipated. Given the dog with severe anemia and a bile fistula we introduce 30 grams of dog hemoglobin and we recover it all or its equivalent as new hemoglobin in red cells. Does this mean no excess of bile pigment elimination above control periods? Actually we observe an excess of bile pigment equivalent to two-thirds or more of the injected hemoglobin.

How may we explain this paradox? We may choose to believe that from the injected dog or sheep hemoglobin the pigment radicle or pyrrol nucleus is split off as usual and utilized to form excess bile pigment in the usual ratio—40 mgm. bile pigment = 1 gram hemoglobin. There is a modest conservation of this pigment radicle as not all of it appears as bile pigment and we may imagine that this small fraction may be recast to form new hemoglobin.

What about all the new hemoglobin which is formed so abundantly above the control level? It would appear that substances other than the pyrrol nucleus and present in the *globin fraction of hemoglobin* were important determining factors in the fabrication of new hemoglobin. If this is so we must postulate an active production of the pyrrol nucleus within the body. Heretofore this possibility has not received serious consideration but if the body can produce the pyrrol nucleus in one emergency it is probable that it does so on other occasions.

Methods. Method details are given in the preceding paper and apply to all the experiments tabulated below. The technique used in the anemic dog has been described by Whipple and Robscheit-Robbins (3). The importance of blood volume determinations has been stressed and is the only method of accurate control of the hemoglobin level in severe anemia. It is obvious that blood concentration may give a false value for the hemoglobin level and without blood volume measurement this error escapes detection. These blood volume determinations were done at the beginning and end of the hemoglobin injection periods and at such other times as considerable amounts of blood were removed to reduce the blood hemoglobin content to the control level. The formula for estimating the total hemoglobin removed by bleeding is given in a recent publication (4). A definite amount is allowed as a base line hemoglobin production (2 grams per week) on the standard salmon bread diet as this is the average hemoglobin production as found in a very large number of anemia controls. This explains why the figures given in the tables which represent actual hemoglobin removal by bleeding do not correspond exactly to the *net total hemoglobin production* as figured in the summary tables and in the discussion of various tables. Care was taken to exhaust the stored reserve which the

body can call out in the early weeks of the severe anemia (4) from which store much hemoglobin can be produced.

Because of its importance we reiterate that these dogs in spite of an experimental anemia due to continued bleeding were in excellent clinical condition and maintained a uniform weight curve.

EXPERIMENTAL OBSERVATIONS. The unexpected trend of these observations necessitates a detailed tabulated record of these experiments. In general the experiments are entirely in accord and seem to establish convincingly that the introduced hemoglobin contributes as usual to the rebuilding of new hemoglobin in anemia with 100 per cent conservation. At the same time there is a large excess of bile pigment excreted above the control level which can be explained only as due to the hemoglobin given intravenously. It would seem that the injected hemoglobin was split into the pyrrol nucleus which appears largely as bile pigment surplus and into some globin fraction or fractions which go to form as much new hemoglobin in new red cells as was injected as laked hemoglobin. This goes on whether dog or sheep hemoglobin is used which indicates obviously an initial katabolism as mentioned.

Table 21 gives a satisfactory experiment to show the result of hemoglobin injection in a renal fistula dog under anemic conditions. We refer to the same dog in table 2 of the first paper for the control. During *non-anemic* periods it will be recalled that his daily bile pigment output was higher than the average—124 mgm. bilirubin. During the *anemic* control periods the bile pigment output falls to 78 mgm. per 24 hours although the general condition is excellent and there is a slight gain in weight.

This dog was included in the anemia colony and the usual routine followed. The amount of new hemoglobin produced and removed by bleeding was estimated by the formula described recently (4). This dog with established stable anemia of low hemoglobin level received 32 grams of dog hemoglobin and during the next two weeks put out 37.2 grams. The average return in anemic dogs without the bile fistula is 90 to 100 per cent recovery.

While this was going on we note the expected increase in bile pigment output—an increase corresponding to 95 per cent of the theoretical return, estimating 1 gram hemoglobin as equivalent to 40 mgm. bilirubin. Therefore we have an increase in bile pigment, presumably due to the splitting off of the pyrrol nucleus and an increase in new hemoglobin production obviously in part due to something other than the pyrrol nucleus, perhaps some fraction of the globin of the injected hemoglobin.

It will be noted that the plasma volume is unchanged by the bleeding. This dog during the hemoglobin injection period was a little upset and his appetite a bit off. For this reason his diet was modified a little from time to time but we do not think it necessary to give all the details. His basal

ration was salmon bread 325 grams, canned salmon 100 grams, Klim 50 grams and dog bile 50 cc. At times 50 grams meat scraps were added to the diet and this may have been responsible for the slight excess hemoglobin

TABLE 21

Bile pigment and hemoglobin recovery after hemoglobin injection

Dog 29–34, renal fistula, anemic

DATE	BILE PIGMENT OUTPUT 24 HRS.	HEMOGLOBIN INJECTION 24 HRS.	HEMOGLOBIN RECOVERY BY BLEEDING	BLOOD HEMOGLOBIN LEVEL	BLOOD PLASMA VOLUME	URINE BILE COLLECTION	WEIGHT
	mgm.	*grams*	*grams*	*per cent*	*cc.*	*cc.*	*kgm.*
July 16	81	0	0.7	41		690	18.7
July 17	87	0				770	18.8
July 18	79	0				605	18.5
July 19	92	0				640	18.2
July 20	93	0				485	
July 21	89	0	1.7	39		470	
July 22	71	2			956	385	17.3
July 23		2					17.3
July 24	142	2				485	17.8
July 25	162	2				1,050	18.0
July 26	138	2				640	18.0
July 27	151	2				780	
July 28	151	2	0.9	58		470	17.8
July 29	130	2				720	18.0
July 30	169	2				820	18.0
July 31	163	2				610	18.0
August 1	134	2				585	17.3
August 2		2					
August 3	112	2				325	17.3
August 4	248	2	0.8	53		705	17.2
August 5	152	2				960	17.6
August 6	154	2				715	18.0
August 7	144	0				950	18.5
August 8	88	0				1,050	18.4
August 9	82	0				955	18.2
August 10	56	0				630	
August 11	80	0		70		1,050	18.5
August 12	77	0	16.1		972	1,165	18.8
August 13	63	0	12.5			805	19.0
August 14	62	0				750	19.0
August 21			14.9	40			18.7

production. At other times meat extract, 5 grams, was used. This is known to be inert as regards hemoglobin regeneration.

Table 22 shows an interesting experiment on a dog which had been anemic more than 2 years. The bile fistula operation was done December

13, 1926. The dog was included in the anemia colony and kept anemic as indicated continuously until the tabulated observations in 1929. He remained in good condition during all this time and we have all his reactions to various diets as well as to hemoglobin injections. During this experiment (table 22) the dog was on a diet consisting of salmon bread 400 grams, canned salmon 100 grams, Klim 25 grams and 40 cc. dog bile.

There are no observations on the bile pigment output of this dog prior

TABLE 22

Bile pigment and hemoglobin recovery after hemoglobin injection

Dog 26–19, renal fistula, anemic

DATE	BILE PIGMENT OUTPUT 24 HRS.	HEMOGLOBIN INJECTION 24 HRS.	HEMOGLOBIN RECOVERY BY BLEEDING	BLOOD HEMOGLOBIN LEVEL	BLOOD PLASMA VOLUME	URINE BILE COLLECTION	WEIGHT
	mgm.	*grams*	*grams*	*per cent*	*cc.*	*cc.*	*kgm.*
April 21	53	0				1,070	14 8
April 22	62	0	1.4	49	990	1,230	14 8
April 23	38	0				960	14 2
April 26	73	0	1.3	45	966	1,000	14 9
April 27	56	0				750	15 5
April 28	77	3				1,160	15.1
April 29	183	3	0.8	52		900	15 0
April 30	157	3				1,030	15.1
May 1	188	3				850	15.0
May 2	162	3				1,040	15 2
May 3	184	3				1,160	15.5
May 4	180	3				1,010	15.4
May 5	173	3				1,120	
May 6	192	3	0.9	60		1,090	15.2
May 7	191	3				810	
May 8	204	3				1,010	
May 9	206	0				870	14.9
May 10	89	0	15.6			1,000	15 5
May 11	81	0				1,030	15.3
May 12	71	0				1,350	
May 13	62	0	12 6	53		1,240	15.1
May 14	58	0				820	15.4
May 23			14.4	52	968		15 3

to the anemia period but during the anemic period he shows a daily output of 65 mgm. bilirubin. The hemoglobin injections raise this level to 184 mgm. per day, a total excess which amounts to 98 per cent of the theoretical return, estimating 1 gram hemoglobin = 40 mgm. bilirubin—consult table 26 below.

This dog (table 22) presents an anemic period of over 2 years with careful standardization in our anemia colony. We feel great confidence there-

fore in the hemoglobin recovery of 95 per cent of the total hemoglobin injection, which corresponds to many observations in anemic dogs without bile fistulas (2). The blood plasma volume is unchanged and there are no confusing factors.

Several weeks after this experiment was completed this dog developed a disturbance observed at times in bile fistulas of the several types. He developed a hemorrhagic tendency, bleeding from needle punctures and mucous surfaces and was killed on July 8, 1929. We hope to report in detail on this interesting condition in the near future.

Autopsy (table 22, dog 26–19) done soon after death July 8, 1929, showed only a few points of interest relating to these experiments. The bile passages were in good order, not dilated nor infected. The renal pelvis, ureter and bladder were also normal. The ribs were soft and could be fractured easily between the fingers. Histological sections show that all the large and small bile ducts are normal. There are no mononuclears in any portal tissue, which shows that there had been no infection on earlier occasions. An occasional bile canaliculus contains a little hyaline cast of brown material probably dating from the long continued hemoglobin injection. The spleen and lymph glands show slight amounts of yellow granular pigment which also probably dates back to former hemoglobin injections. The kidneys are normal. The bone marrow shows the usual extreme hyperplasia found in this type of long continued anemia due to bleeding. There are no other abnormalities of interest relating to these experiments.

Table 23 presents another complete experiment during anemia and we may refer to the non-anemic control period—see table 1 in preceding paper. This dog was operated upon on May 1, 1929, and the first hemoglobin injection experiment (table 1) done in July and August. The average daily output of bile pigment amounts to 57 mgm. during the normal periods on a salmon bread standard ration.

After anemia had been produced, 3 months later we record the data in table 23 and note that the daily output falls to 28 mgm. bilirubin under standard diet conditions with an anemia level of less than one-third normal control blood hemoglobin levels. With an anemia level of 30 per cent normal we see a bile pigment output level of 50 per cent normal, an indication that muscle hemoglobin may contribute to the bile pigment output—see discussion below.

Table 23 shows an unusually long period of hemoglobin injection and the control fore and after periods amount to the same number or 18 days, see table 27. In table 23 we omit the first 3 control days and the last 3 control days but these are included in the general averages as given below. The total hemoglobin injection is 36 grams freshly prepared dog hemoglobin and we note a recovery of 31.3 grams hemoglobin by bleeding to adjust

the anemia level to the pre-injection level. This makes a recovery of 87 per cent of the injected dog hemoglobin as new formed red cells.

Table 23 shows also that the total output of bilirubin resulting from this hemoglobin injection amounts to 1133 mgm. above the control level.

TABLE 23

Bile pigment and hemoglobin recovery after hemoglobin injection

Dog 26–45, renal fistula, anemic

DATE	BILE PIGMENT OUTPUT 24 HRS.	HEMOGLOBIN INJECTION 24 HRS.	HEMOGLOBIN RECOVERY BY BLEEDING	BLOOD HEMOGLOBIN LEVEL	BLOOD PLASMA VOLUME	URINE BILE COLLECTION	WEIGHT
	mgm.	*grams*	*grams*	*per cent*	*cc.*	*cc.*	*kgm.*
November 14	31	0		41		735	12.0
November 15	24	0				580	11.6
November 16	35	0				575	11.4
November 17	43	0				600	11.9
November 18	28	0				475	11.7
November 19	31	0		41	754	580	11.5
November 20		2					11.5
November 21	54	2				635	11.5
November 22	78	2				725	11.8
November 23	93	2				835	11.9
November 24	102	2				1,290	12.2
November 25	78	2				685	11.9
November 26	86	2				545	11.9
November 27	109	2		49	726	730	11.8
November 28	105	2				685	11.8
November 29	98	2				560	11.8
November 30	77	2				730	12.1
December 1	87	2				730	12.1
December 2	77	2				615	12.0
December 3	103	2				1,000	12.3
December 4	92	2		57	736	520	12.0
December 5	99	2				525	12.0
December 6	135	2				875	12.4
December 7	77	2				660	11.9
December 8	87	0				690	11.7
December 9	31	0	14.2	75		540	12.0
December 10	13	0	10.8			350	12.0
December 11	14	0	7.3			485	11.8
December 12	19	0		40	645	500	11.7
December 13	27	0				570	11.7

This is equivalent to 28.4 grams hemoglobin according to the accepted formula or 79 per cent of the pigment radicle contained in the injected hemoglobin—consult also table 27.

During this experimental period (table 23) this dog received a daily

ration as follows—salmon bread 250 grams, canned salmon 100 grams, Klim 50 grams, Lemco meat extract 10 grams, Bacto ox bile 8 grams in place of whole dog bile. This was mixed into a mash and well eaten as indicated by the weight curve.

Two similar experiments to that tabulated in table 23 were done with this same dog 26–45 following this experimental period. The same general results were forthcoming and are given in summary in tables 26 and 27 below. After these three experiments with dog hemoglobin we record a complete experiment with *sheep hemoglobin* in this same dog—dog 26–45, table 24.

TABLE 24

Bile pigment and hemoglobin recovery after sheep hemoglobin injection

Dog 26–45, renal fistula, anemic

DATE	BILE PIGMENT OUTPUT 24 HRS.	HEMOGLOBIN INJECTION 24 HRS.	HEMOGLOBIN RECOVERY BY BLEEDING	BLOOD HEMOGLOBIN LEVEL	BLOOD PLASMA VOLUME	URINE BILE COLLECTION	WEIGHT
	mgm.	*grams*	*grams*	*per cent*	*cc.*	*cc.*	*kgm.*
April 26	33	0				560	12.0
April 27	32	0				535	
April 28	36	0	1.2	42	747	500	
April 29	30	0				620	
April 30	25	0				630	
May 1	27	0.6				500	
May 2	43	1.0				440	
May 3	52	1.0				685	
May 4	36	1.0				685	
May 5		1.0					11.9
May 6	61	1.2	0.6	51		720	
May 7	63	1.0				705	
May 8	63	1.0				810	12.3
May 9	61	1.0				665	
May 10	60	1.0				750	
May 11	74	1.0				830	
May 12	55	1.0	0.8	55		835	
May 13	64	0				685	12.2
May 14	46	0				765	12.4
May 15	32	0				605	
May 16	40	0				735	
May 17	36	0				710	
May 18	29	0				595	
May 19	31	0	1.4	50	697		
June 3			24.4	40			11.8

Table 24 shows the same general results with *sheep hemoglobin injection* as with dog hemoglobin in other tables. It is not safe to give very large

doses of sheep hemoglobin and these large doses would be unsatisfactory for this type of experiment because the renal threshold for sheep hemoglobin is about half that for dog hemoglobin and much of the injected hemoglobin would escape unchanged in the urine. In these experiments there was no escape of hemoglobin in the urine.

In all, this dog received 11.8 grams of sheep hemoglobin intravenously and was bled 16.2 grams of new hemoglobin to adjust his anemia level to the fore period level. This indicates about 100 per cent conservation of the injected hemoglobin as is observed in other experiments. The slight increase of 4 grams in the hemoglobin removed above the hemoglobin injected is within the method error of such experiments but there is ample evidence here and elsewhere that sheep hemoglobin is carefully conserved when given intravenously in anemic dogs and recast into new dog hemoglobin.

The bile pigment surplus over control periods amounts to a total of 292 mgm., the equivalent of 7.3 grams of hemoglobin or a recovery ratio of 60 per cent. The dog was not disturbed by these injections and presents a uniform weight curve. During this experimental period this dog received the following daily diet which was well eaten—salmon bread 250 grams, canned salmon 100 grams, Klim 50 grams, dog bile 50 cc.

The last experiment on dog 26–45 is given in table 25. It will be noted that through all these months this dog has remained in excellent clinical condition in spite of the severe anemia. The weight remains constant and there are no complications of any kind. Moreover it is of interest to note that the control bile pigment daily output averages 28 mgm. which is identical with the control level 8 months previously—see table 23.

The total dog hemoglobin given intravenously amounts to 32 grams and the recovery of new hemoglobin from bleeding is 40.2 grams which shows new hemoglobin production in excess of the average return. This is probably explained by the diet which during this period was made up as follows—salmon bread 250 grams, canned salmon 100 grams, Klim 50 grams, bile 50 cc. and meat scraps 50 grams. The meat scraps were given to increase the palatability of the daily diet.

The increase in bile pigment amounts to a total of 630 mgm. above the control level (tables 25 and 27) which is equivalent to 15.7 grams hemoglobin and a recovery ratio of 49 per cent. It is of course possible if not probable that some of this pigment radicle not appearing as surplus bile pigment did actually contribute to the formation of new blood hemoglobin.

Table 26 gives a summary of seven experiments which are quite uniform in respect to bile pigment elimination and hemoglobin conservation following intravenous hemoglobin injection as indicated. We note that the recovery ratio of bile pigment coming from hemoglobin injection averages

68 per cent—corrected for 10 per cent loss due to method we get 75 per cent recovery which is to be compared with the figures in non-anemic dogs

TABLE 25

Bile pigment and hemoglobin recovery after dog hemoglobin injection

Dog 26–45, renal fistula, anemic

DATE	BILE PIGMENT OUTPUT 24 HRS.	HEMOGLOBIN INJECTION 24 HRS.	HEMOGLOBIN RECOVERY BY BLEEDING	BLOOD HEMOGLOBIN LEVEL	BLOOD PLASMA VOLUME	URINE BILE COLLECTION	WEIGHT
	mgm.	*grams*	*grams*	*per cent*	*cc.*	*cc.*	*kgm.*
July 10	33	0	0.5	39	784	620	11.4
July 11	36	0				520	11.1
July 12	31	0				610	11.2
July 13	20	0				470	
July 14	47	0	0.6	44		465	10.9
July 15	27	0				525	10.9
July 16	22	0				890	11.3
July 17	18	2				955	11.4
July 18	37	2				775	11.7
July 19	54	2				695	11.9
July 20	55	2				905	
July 21	58	2	0.6	43		700	
July 22	95	2				890	11.5
July 23	45	2				700	
July 24	69	2				990	12.1
July 25	88	2				1,180	12.1
July 26	57	2				510	11.9
July 27	101	2				835	11.8
July 28	68	2	1.1	58		815	
July 29	59	2				995	12.2
July 30	90	2				1,150	12.0
July 31	69	2				1,010	12.1
August 1	60	2				625	12.0
August 2	75	0				830	12.3
August 3	29	0				780	12.4
August 4	27	0	16.8	68	685	640	12.5
August 5	39	0				895	12.4
August 6	31	0	13.1			755	12.5
August 7	26	0				635	12.4
August 8	18	0	0.7	55		735	12.4
August 9	28	0				745	12.4
August 10	18	0			689	700	
August 18			27.9	45			

—recovery of 87 per cent corrected for method loss giving 96 per cent recovery. It is noted in table 26 that dog 26–45 gives lower values for bile pigment elimination than the other two dogs and this dog also has a low

daily output of bile pigment in all control periods. We are unable to offer a satisfactory explanation for these observations.

Table 26 also shows the conservation of injected hemoglobin for all experiments including sheep hemoglobin as indicated by the hemoglobin removed by bleeding to adjust the blood hemoglobin level to the control level of the fore period. Such figures in anemic standardized dogs average between 90 and 100 per cent and in these anemic bile fistula dogs the same thing holds. Three experiments show a little more hemoglobin removed than was injected as laked hemoglobin. We believe these surpluses are to be explained in part by the diet and in part by method limitations. There is no convincing evidence that the bone marrow is stimulated to unusual activity under these conditions but it is difficult to establish this point one way or another.

SUMMARY TABLE 26

Bile pigment and hemoglobin recovery after hemoglobin injection in anemia

DOG	BILIRUBIN OUTPUT AVERAGE PER 24 HRS.					HEMOGLOBIN PER 24 HRS.		
	Fore period control	Hemoglobin injection period	After period control	Excess due to hemoglobin injection	Recovery ratio from hemoglobin injection	Average injection	Recovery average	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*grams*	*grams*	*per cent*
26-19	60	184	72	118	98	3	2.84	95
26-45	31	91	25	63	79	2	1.74	87
26-45	29	61	22	36	44	2	1.86	93
26-45	36	78	33	44	54	2	1.42	71
26-45	29	68	27	40	49	2	2.51	125
26-45	31	58	36	24	60	1	1.35	137
29-34*	83	154	73	76	95	2	2.33	116

One gram hemoglobin equivalent to 40 mgm. bile pigment.

* Sheep hemoglobin used in place of dog hemoglobin.

Table 27 summarizes the experimental data to give total values for the removed hemoglobin and surplus bile pigments. This table is frequently referred to in discussion of individual experiments given above.

Discussion. The life cycle of the circulating red cell has been calculated frequently on the basis of the bile pigment output. The errors inherent in any such calculation have been pointed out elsewhere (5). It is necessary to note only that muscle hemoglobin is broken down into bile pigments and it seems safe to assume that muscle hemoglobin contributes to the bile pigment excretion. In the dog the muscle hemoglobin may be no inconsiderable part of the total body hemoglobin. For example, in the normal dog (6) we may find that the muscle hemoglobin may amount to 15 or 25 grams if we represent the total circulating hemoglobin as 100 grams. In an active muscular dog made anemic as in these experiments

we may find 80 grams muscle hemoglobin if we represent the total circulating hemoglobin as 100 grams. This might mean that in an active dog made anemic the *muscle hemoglobin* might contribute a sizeable portion of the total observed daily output of bilirubin.

It is interesting to speculate on this point when we subject certain figures to analysis—see table 28.

We observe (table 28) that in dog 26–45 the hemoglobin level is reduced to $\frac{1}{3}$ normal but the bile pigments fall to $\frac{1}{2}$ normal. In dog 29–34 the hemoglobin level is reduced to $\frac{1}{2}$ normal and the bile pigments fall to $\frac{3}{5}$ normal. As a matter of fact, with anemia the total bulk of circulating red cells is decreased to lower values than are the figures for blood hemoglobin. This is due to a slight decrease in the total blood volume. There is moder-

SUMMARY TABLE 27

Total hemoglobin injection and total recovery of bile pigment and hemoglobin in anemia

DOG		TOTAL HEMOGLOBIN			TOTAL BILE PIGMENT ABOVE CONTROL LEVEL	BILE PIGMENT EXCESS ABOVE CONTROL	
Number	Weight	Intake intravenous	Output bleeding	Output-intake ratio		Equivalent to hemoglobin	Expressed as per cent of hemoglobin injection
	kgm.	*grams*	*grams*	*per cent*	*mgm.*	*grams*	
26-19	15.1	33	31.1	95	1,300	32.5	98.0
26-45	11.7	36	31.3	87	1,133	28.4	79.0
26-45	11.5	32	29.7	93	564	14.1	44.0
26-45	12.0	32	22.8	71	689	17.2	54.0
26-45	12.0	32	40.2	125	630	15.7	49.0
26-45*	12.0	12	16.2	137	292	7.3	60.0
29-34	18.3	32	37.2	116	1,060	26.5	95.0

One gram hemoglobin is equivalent to 40 mgm. bilirubin.

* Sheep hemoglobin used in place of dog hemoglobin.

ate increase in the plasma volume in anemia but not enough to bring the blood volume up to its non-anemic volume. This actually increases the discrepancy between the blood hemoglobin levels and bile pigment output level. If we compare these analyses we find in round numbers (dog 26–45) that it is possible to argue that the muscle hemoglobin contributes 15 mgm. of the total bile pigment. In anemia 15 mgm. from muscle hemoglobin + 15 mgm. from blood hemoglobin equals the total 30 as given. In normal conditions 15 mgm. from muscle hemoglobin + 15 × 3 (to allow for normal hemoglobin level) equals 60 or 57 as given.

In the same way we may figure dog 29–34 for example 25 mgm. as coming from muscle hemoglobin and 50 mgm. from the blood hemoglobin in anemia or 100 mgm. in normal periods giving the total bile pigment as tabulated. The muscle hemoglobin is not changed by anemia periods and

by inactivity only moderately. It is a very stable muscle constituent in the adult dog (6). The dog 29–34 weighs 18 kgm. as compared to the dog 26–45 weighing 12 kgm. These two figures for muscle hemoglobin and bile pigment production 25 and 15 mgm. respectively come out fairly well as compared with the weight which in an active dog gives a good approximation of the volume of muscle hemoglobin.

This analysis need not be carried too far but from these figures, if we are forced to admit considerable amounts of bilirubin may come from muscle hemoglobin, we thereby add many days to the estimated life cycle of the red cell in the circulation of the healthy dog. For example, we may argue that dog 26–45 with a weight of 12 kgm. and a plasma volume of 700 cc., a low anemia level of 45 per cent and red cell hematocrit of 20 per cent, would have in his circulation ($8.75 \times 0.45 \times 13.8$) 56 grams hemoglobin. If his output averages 2.5 mgm. bile pigment per kilo per 24 hours, this might mean $\frac{3}{4}$ gram hemoglobin destroyed a day, but if in anemia only one-half of this is derived from blood hemoglobin we would arrive at 150

COMPARISON TABLE 28

	DOG 29–34		DOG 26–45	
	Bilirubin output	Blood hemoglobin level	Bilirubin output	Blood hemoglobin level
	mgm.	*per cent*	*mgm.*	*per cent*
Normal level	124	120	57	140
Anemic level	78	60	30	45

Dog 29–34 = 18 kgm. weight. Dog 26–45 = 12 kgm. weight.

days for the life cycle of the red cell under these conditions. We do not propose this seriously but give the figures as they stand, to indicate the type of argument which finds favor with some workers.

We have a conviction that under a variety of circumstances the liver may contribute from its store to the fabrication of blood hemoglobin pigment or bile pigment. We may say the liver has fluid assets (capacity of synthesis) which may be used to form pigments or other materials depending upon the demands of the moment. This conception does not simplify the story of pigment metabolism but possibly study in this field has been confused by the simple if not infantile concepts which have become so firmly fixed in the literature of this subject.

SUMMARY

A severe experimental anemia due to bleeding may be combined with a renal-bile fistula and enable the investigators to follow simultaneously new hemoglobin production and bile pigment output under carefully controlled conditions. Dogs can be kept in perfect physical condition with

normal activity and uniform weight under these conditions provided care is taken to insure suitable diet intake.

Under such conditions the base line control output of bile pigments per 24 hours is much less than during non-anemic periods. This bile pigment output does not fall as low in proportion as does the "anemia" level—for example, with an anemia level of one-third normal hemoglobin, we observe a bile pigment output level of one-half normal.

This pigment paradox reads about as follows—from the injected hemoglobin comes a quantitative return of new hemoglobin in red blood cells removed by bleeding. At the same time there is *almost a quantitative return from the same hemoglobin injection* in the form of bile pigment. Whatever pigment is accepted as the end product of the introduced hemoglobin, we face the necessity of explaining the other pigment formation.

We suggest as a possibility that from the injected hemoglobin is split off the pyrrol nucleus to form bilirubin and from the *globin fraction* or parts of it may come much of the new hemoglobin which appears in the circulating red cells. If this is true it appears that the body can *synthesize* the *pyrrol nucleus* in considerable amounts in an emergency due to anemia.

There is urgent need for much careful study of the globin fractions.

BIBLIOGRAPHY

(1) WHIPPLE AND HOOPER. This Journal, 1917, xliii, 258.
(2) WHIPPLE, G. H. AND F. S. ROBSCHEIT-ROBBINS. This Journal, 1927, lxxxiii, 60.
(3) WHIPPLE, G. H. AND F. S. ROBSCHEIT-ROBBINS. This Journal, 1925, lxxii, 395.
(4) WHIPPLE, G. H. AND F. S. ROBSCHEIT-ROBBINS. This Journal, 1930, xcii, 362.
(5) WHIPPLE, G. H. Physiol. Reviews, 1922, ii, 440.
(6) WHIPPLE, G. H. This Journal, 1926, lxxvi, 693.

FURTHER EXPERIMENTS ON DIRECT AND INDIRECT SCN-CONTRACTURE

The Dependence of Ion Antagonism on Permeability

E. GELLHORN

From the Department of Animal Biology, University of Oregon, Eugene, Oregon

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In a previous paper in which "indirect" SCN contracture was studied, (3) it was shown that the immersion of the sartorius in a Ringer's solution containing NaSCN[1] the concentration of which is below that causing contracture has no direct effect on contracture, it however gives rise to a contracture, when this solution is replaced by common Ringer's solution.[2] The determination of the isometric tension of this contracture, and the application of different SCN-salts proved that it is an SCN contracture. The hypothesis was made that SCN penetrates the cell rather slowly and therefore does not produce an effect in the first solution, but SCN gradually increases permeability of the muscle when it is in contact with it for a sufficient length of time. Consequently when the first solution is exchanged for the second (SCN-free) solution, SCN leaves the cell rapidly and thus gives rise to the indirect SCN contracture.

If the hypothesis is correct it is to be expected that changes in permeability of the cell influence the magnitude of the indirect contracture. This has been partially proved in the first paper in which the composition of the second solution was varied. Increased permeability produced an increase in the magnitude of the indirect contracture while decrease of permeability had the opposite effect.

A further consequence of the hypothesis made above is:

1. That a change in permeability produced by the first solution should produce a corresponding change in the magnitude of indirect contracture.

2. That the efficiency of ions in the first and second solutions should be different on account of the different degree of permeability of the muscle.

3. That the time of immersion in the first solution should greatly influence indirect contracture because changes in permeability occur slowly.

The experiments described in this paper show, in fact, the correctness of the supposition. Further experiments deal with a comparison of direct and indirect contracture.

[1] First solution.

[2] Second solution.

I. *The importance of the time of application of the first solution for the magnitude of the indirect contracture.* It is known that changes in permeability take a measurable time, as v. Gulacsy has recently shown for changes in the permeability of muscle resulting from electrical stimulation. The following experiments favor the opinion that this is true also for changes

TABLE 1

NUMBER	FIRST SOLUTION	SECOND SOLUTION*	DURATION OF IMMERSION IN FIRST SOLUTION	HEIGHT OF** INDIRECT CONTRACTURE IN SECOND
				mm.
1	9.5 cc. NaSCN*** + 7.5 cc. NaCl	12 cc. Ringer + 5 cc. NaCl	0.5 minute	0
	" "	" "	1.5 minutes	7
	" "	" "	4.0 minutes	43
2	9.0 cc. NaSCN + 7.0 cc. NaCl	10 cc. Ringer + 6 cc. NaCl	1.5 minutes	0
	" "	" "	3.0 minutes	15
	" "	" "	4.0 minutes	20.5
3	9.0 cc. NaSCN + 7.0 cc. NaCl	12 cc. Ringer + 4 cc. NaCl	1.0 minute	6
	" "	" "	2.0 minutes	9
	" "	" "	3.0 minutes	19
4	13.0 cc. Ringer + 3.0 cc. NaSCN	16 cc. NaCl	84 seconds	45
		16 cc. NaCl	15 seconds	29
5	10.0 cc. Ringer + 6.0 cc. NaSCN	16 cc. NaCl	20 seconds	38
	" "	16 cc. NaCl	91 seconds	62
6	15.0 cc. Ringer + 1.0 cc. NaSCN	16 cc. NaCl	98 seconds	14
	" "	16 cc. NaCl	16 seconds	1.8

* When the muscle is taken from the first and put into the second solution the indirect contracture takes place.

** The magnification through the lever has not been considered because the numbers have only relative value.

*** The salt solutions were isotonic. ($\Delta = -0.39°$)

in permeability produced by chemical reagents. In table 1 experiments are reproduced in which the time of immersion in the first solution was varied. The table shows that there is a close parallelism between the time of immersion and the strength of the indirect contracture. It is remarkable that in some cases (expts. I and II), when the solutions were applied for 0.5 minute and 1.5 minute respectively they were ineffective, but when

they were applied for a longer time the reaction took place. We conclude from these observations: 1, that during immersion in the solution containing NaSCN the permeability of the muscle increases; 2, that the maximum of permeability is not reached even after 2 or 3 minutes; 3, that other conditions being equal indirect contracture is stronger the more the permeability in the first solution is increased.

II. *Permeability and ion effects in indirect NaSCN contracture.* That changes in the permeability of a muscle during the time of immersion in the

TABLE 2

NUMBER		FIRST SOLUTION	SECOND SOLUTION	HEIGHT OF THE INDIRECT CONTRACTURE
				mm.
1	a	9 cc. NaSCN 10 cc. Ringer	19 cc. NaCl	44
	b	9 cc. NaSCN 9 cc. Ringer 1 cc. $CaCl_2$	19 cc. NaCl	21
	c	9 cc. NaSCN 10 cc. Ringer	18 cc. NaCl 1 cc. $CaCl_2$	0
	d	9 cc. NaSCN 10 cc. Ringer	19 cc. NaCl	37
2	a	9 cc. NaSCN 10 cc. Ringer	19 cc. NaCl	25
	b	9 cc. NaSCN 8 cc. Ringer 2 cc. $MgCl_2$	19 cc. NaCl	7
	c	9 cc. NaSCN 10 cc. Ringer	17 cc. NaCl 2 cc. $MgCl_2$	1
	d	9 cc. NaSCN 10 cc. Ringer	19 cc. NaCl	25

first solution influence indirect contracture can be proved by further experiments in which the physico-chemical composition of the first solution is changed. A typical experiment is reproduced in figure A. In this experiment the influence upon the indirect contracture of $CaCl_2$ added to the first solution was determined. It is evident that the strength of the indirect contracture in the first and third experiment is about the same, which proves the complete reversibility of the $CaCl_2$ effect studied in the second experiment. This experiment is similar to those formerly described in which $CaCl_2$ gave the same effect if added to the second solution.

Further experiments showed that other conditions being equal indirect contracture decreases if $SrCl_2$ or $MgCl_2$ are added. In other words: ions which reduce indirect contracture when added to the second solution, exert the same effect in the first. But in spite of the qualitative agreement

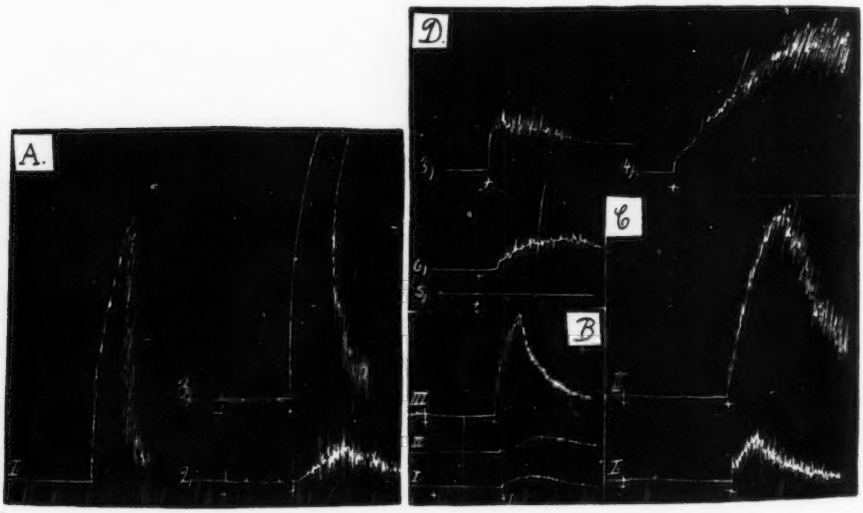

Fig. A. Expts. I and III. At the first +, Ringer was exchanged for : 4 cc. NaSCN + 12 cc. Ringer; at the second +, 16 cc. NaCl replaced Ringer. Expt. II At the first +: 4 cc. NaSCN + 4 cc. $CaCl_2$ + 8 cc. Ringer. At the second +: 16 cc. NaCl.

Fig. B. Expt. I At the first +: 9 cc. Ringer + 9 cc. NaSCN. At the second +: 18 cc. Ringer. Expt. II At the first +: 18 cc. NaSCN. At the second +: 18 cc. Ringer. Expt. III At the first +: 5 cc. NaSCN + 13 cc. Ringer. At the second +: 18 cc. NaCl.

Fig. C. Expt. I At the first +: 14.5 cc. Ringer + 0.5 cc. NaSCN. At the second +: 15 cc. NaCl. Expt. II At the first +: 8 cc. Ringer + 7 cc. NaSCN. At the second +: 15 cc. NaCl.

Fig. D. Experiments on direct NaSCN-contracture. At + Ringer was exchanged for: 9 cc. NaSCN + 9 cc. NaCl (expt. 3); 9 cc. NaSCN + 8.5 cc. NaCl + 0.5 cc. $BaCl_2$ (expt. 4); 9 cc. NaSCN + 9 cc. Ringer (expt. 5); 9 cc. NaSCN + 8.5 cc. Ringer + 0.5 cc. $BaCl_2$ (expt. 6).

there is a remarkable quantitative difference. In general the indirect contracture can be influenced much more easily through a change in the second than in the first solution. With smaller amounts of ions greater effects are obtained in the second solution. This may be illustrated by table 2.

The fact that the indirect contracture can be influenced better through a change in the second solution than in the first was frequently observed. If, for example, a muscle was not very sensitive to the indirect contracture, better results were obtained by making a small change in the second solution, for example, by using NaCl instead of Ringer's, than by increasing the NaSCN concentration in the first solution. This may be demonstrated in figure B in which with Ringer's as the second solution almost no change in the strength of contracture was obtained although the concentration of NaSCN was doubled in the first solution. But by using NaCl instead of Ringer's as the second solution a strong contracture was obtained even with a very low concentration of NaSCN (expt. III). If, however, the conditions were favorable—e.g., no Ca in the second solution—the influence of different concentrations of NaSCN was easily demonstrated, figure C.

It seems to be of general interest for the physiology of the cell that the muscle shows qualitatively the same, but quantitatively very different reactions according to whether a salt is added to the first or the second solution. Because the effects of the ions in question are due to their influence upon the surface of the muscle, it is probable that a change in the behavior of this surface leads to different reactions of the cell to the same ion.

Now the facts supporting the assumption that the muscle is in a state of very high permeability when the first solution is exchanged for the second one were described in a previous paper. One might conclude that a change in the permeability of the cell may lead to a change in the quantitative reactivity of the cell for ions. This hypothesis is supported by other experiments which were reported in a preliminary note (1930). It could be shown that the reactivity of stimulated muscle to ions (e.g., Ca) is quite different from that of resting muscle. The optimal solution for the resting muscle is not the best for the stimulated one. In other words: irritability and fatigability of the muscle show a different dependence upon the ions. These observations are to a certain extent analogous to those upon indirect SCN contracture. For it is known that stimulation of the muscle leads to an increase in permeability. So the experiments of this paper are another example that the reactivity of a cell to ions changes when the permeability of the cell is changed.

III. *Direct NaSCN contracture.* A series of experiments was carried out for the purpose of studying the influence of ions upon the strength of the *direct* NaSCN contracture. Direct contracture is observed in the presence of NaSCN. As explained above the muscle is often not very sensitive to SCN. This gives rise to indirect contracture. But by taking isotonic solutions of NaSCN without the addition of Ringer's solution direct NaSCN contractures are easily obtained. This was rather often

the case too if the solution contained small amounts of Ringer's or isotonic NaCl solution. Thus a basis was given for a study of the behavior of direct contracture. It was found that direct contracture has the same characteristics as the indirect one. It is dependent upon the pH and the osmotic pressure in the same way as indirect contracture. The same holds for the bivalent ions. In figure D a typical experiment is reproduced. It is evident that in the presence of $BaCl_2$ the contracture is increased greatly just as in indirect contracture. Furthermore, in NaSCN + NaCl instead of the same amount of NaSCN + Ringer's the contracture is much greater in the former than in the latter case. In both cases the increase of the contracture in the presence of $BaCl_2$ is demonstrated. The other bivalent ions, e.g., Ca, Sr and Mg, diminish or prevent the direct NaSCN contracture just as they do in the case of the indirect one.

If one compares the behavior of NaSCN-contracture with that of KCl-contracture (1) one sees, with one exception, a close parallelism. While Ba increases the direct and the indirect NaSCN-contracture it decreases the KCl-contracture in the same degree as do the other alkali earths. So far no explanation for this has been found. Further experiments must decide whether or not there is a synergism of more general importance holding for Ba and anions.

Under what conditions does one obtain a direct or an indirect contracture? The answer is in general that one obtains an indirect contracture with lower concentration of NaSCN than is necessary to obtain a direct one. Strong indirect contractures are obtained if the permeability of the muscle is rather low in the first solution in the presence of NaSCN, and high in the second solution which is free of NaSCN. Thus one of the most effective conditions for obtaining the indirect contracture is to use NaSCN in the presence of Ringer's solution and to replace it with pure NaCl solution afterward. The presence of Ca in Ringer's solution reduces the permeability in the first solution thus avoiding direct contracture, while the lack of it in the second solution greatly increases the permeability and thus creates a favorable condition for indirect contracture.

SUMMARY

1. The strength of the indirect SCN-contracture depends very much upon the time during which the solution containing NaSCN is in contact with the muscle. The indirect contracture is also influenced by changing the composition of the solution containing NaSCN. But in general alterations in the second solution for which the NaSCN solution is exchanged influence the muscle more profoundly. There is a remarkable quantitative difference in the susceptibility of muscle to the same ions which seems to be the expression of a more general rule: that ion effects depend to great extent upon the permeability of the cell.

2. There is a close parallelism between KCl-contracture and direct SCN-contracture with respect to the influence of ions, but with the exception that Ba acts upon the KCl-contracture as the other alkali earths do while it has just the opposite effect upon both direct and indirect NaSCN-contracture.

3. The direct and the indirect SCN-contracture in the tissues of vertebrates can be used to demonstrate the laws of permeability.

BIBLIOGRAPHY

(1) GELHORN, E. 1928. Pflüger's Arch., ccxix, 761.
(2) GELLHORN, E. 1930. Contributions to marine biology. Stanford. Univ. Press., p. 115.
(3) GELLHORN, E. 1931. This Journal, xcvi, 203.
(4) v. GULACSY, Z. 1929. Pflüger's Arch., ccxxiii, 407.

STUDIES ON THE ACCELERATION AND INHIBITION OF HEMOLYSIS

II. The Effect of Sugar Solutions on the Resistance of Hypotonic Hemolytic Systems

J. FRANKLIN YEAGER

From Washington Square College, New York University

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The effects of sucrose upon the hemolysis of the red cells of man by the simple lysins, saponin and sodium taurocholate, have been studied and the results reported in a previous paper (1). The present paper is a report of experiments on the effects produced by sucrose and other sugars in various hemolytic systems and suggests a number of factors that should be taken into consideration by any complete description of the process of hypotonic hemolysis of non-nucleated mammalian red cells.

Method. The method of preparing the hypotonic system employed in the present experiments is a slight modification of the method described by Ponder (2). The system is formed by adding together the following components, usually in the order given:

(a) —— cc. M/3.6 sugar
(b) —— cc. 0.80 per cent NaCl
(c) —— cc. distilled water
(d) 0.40 cc. red cell

A standard red cell suspension consists of the cells from 1 cc. of blood suspended in 20 cc. of suspension fluid; a suspension of one-half standard strength is used in these experiments. Before being suspended in 0.80 per cent NaCl or a known mixture of 0.80 per cent NaCl and M/3.6 sugar solution, the cells are washed several times, usually in 0.80 per cent NaCl. Blood drawn into 2 per cent sodium citrate and washed three times in approximately 10 cc. volumes of 0.80 per cent NaCl may be described as a citrate-NaCl-NaCl-NaCl suspension.

For purposes of convenience, 0.80 per cent NaCl has been taken as an "isotonic" salt solution, although some other concentration that does not produce an appreciable amount of hemolysis within the time of the experiment could be used instead. It has been pointed out by Kerr (3) that under certain conditions, such as those which obtain in the present experiments, the membrane of the red cell may be permeable to some extent to

sodium and other cations and that the so-called "isotonic" salt solution may not be truly isotonic, in the sense, for example, that sodium fails to enter the red cells. Furthermore, the exact conditions under which glucose and other sugars enter the red cell appear, from the literature on the subject, to remain in considerable obscurity. The question of red cell permeability to salts and sugars is of interest from the standpoint of experiments to be presented below, which are most readily interpreted on the grounds that certain osmotically active substances penetrate the red cell membrane.

The quantities of components (a), (b) and (c) are so adjusted that the system contains known amounts of 0.80 per cent NaCl and of M/3.6 sugar solution and possesses a known initial tonicity. Table 1 gives the quantities in cubic centimeters of 0.80 per cent NaCl and of distilled water

TABLE 1

0.80 PER CENT NaCl	DISTILLED WATER	INITIAL CONCENTRATION
cc.	*cc.*	*per cent*
0	1.60	0.16
0.15	1.45	0.22
0.30	1.30	0.28
0.45	1.15	0.34
0.60	1.00	0.40
0.75	0.85	0.46
0.90	0.70	0.52
1.05	0.55	0.58
1.20	0.40	0.64
1.35	0.25	0.70
1.50	0.10	0.76
1.60	0	0.80

that are added to the system in order for the latter to have the required initial concentration; the latter is given in the third column of the table as per cent. In calculating the initial concentration of the system no account is taken of the volume occupied by the red cells in the 0.40 cc. of suspension, the latter being considered the same as 0.40 cc. of saline. The above table refers only to those systems that contain zero quantity of component (a); when the latter is an actual quantity it is subtracted from (b). The system having an initial concentration of 0.30 per cent and containing 0.20 cc. of M/3.6 levulose may be taken as an illustrative example; it is formed by adding together

0.20 cc. M/3.6 levulose
0.20 cc. 0.80 per cent NaCl
1.20 cc. distilled water
0.40 cc. 0.5 standard NaCl suspension

In practice, the quantities of levulose, saline and distilled water are added together, in the order given, with a 1 cc. pipette, graduated to 0.01 cc. and brought to constant temperature in a water bath; 0.04 cc. of the red cell suspension, also at constant temperature, is then added. The water bath in an improved form of one previously used (4) and has been described in a previous paper (5). "Time of hemolysis" is the time from the completion of the system by the addition of the cell suspension to the moment at which the lines of the background of the bath become visible when seen through the test tube containing the system. This corresponds to about 90–95 per cent hemolysis. In the determination of the resistance of a system the end-point is that of approximately complete hemolysis.

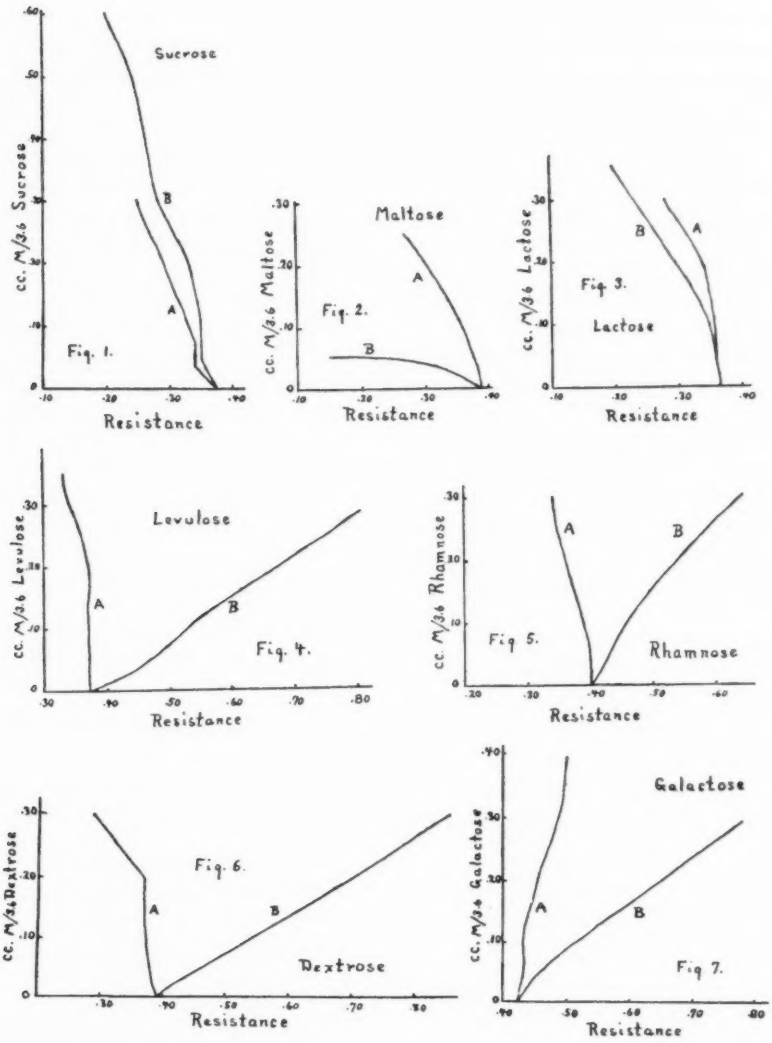
In order to determine the resistance of a system containing zero quantity of a M/3.6 sugar solution, several test tubes are prepared and arranged in an increasing order of initial concentrations. The initial concentration of that tube in which complete hemolysis just occurs in one hour is taken as the resistance of the system. In a similar manner the resistance of systems containing increasing quantities of sugar solution may be obtained. The sugar solution may be added to the system as component (a) or as a part of the cell suspension (d); in either case the addition of a quantity of M/3.6 sugar is necessarily accompanied by the removal of an equal quantity of saline.

Results. In each experiment, done at 25°C., the red cells of man were used.

(a) Changes in resistance due to addition of sugar: changes in the resistance of hypotonic systems have been obtained by the addition of M/3.6 solutions of the following sugars. The results of a typical experiment with each sugar will be given briefly.

Sucrose. Citrate-NaCl-NaCl-NaCl and citrate-NaCl-NaCl-(NaCl-sucrose) suspension of cells were prepared and allowed to stand for three and one-half hours before use. Addition of increasing quantities of sucrose to the hypotonic hemolytic system results in an accompanying increase of resistance, the increase occurring when the quantities of sugar solution are added via the suspension as a portion of the suspension medium. This is an immediate effect, in contrast to the more prolonged effect, which appears as a relative decrease in resistance. The two effects illustrated in figure 1 are quite distinct. The immediate effect is shown by both curve *A* and curve *B*. Curve *B* shows, however, not only the immediate effect of increased resistance but also the more prolonged effect of a resistance somewhat decreased as compared to curve *A*. The comparative decrease of resistance is appreciable in the case of sucrose and is much greater in the case of some of the other sugars. The red cells of the hemolytic system represented by curve *A* first come into contact with the sugar solution when the formation of the system is completed by the addition of cell suspension,

while the cells of the system represented by curve B remain in contact with the sugar solution for some time before the system is formed. This



prolonged exposure of the red cells to the sugar solution in the suspension medium results in the relative decrease in resistance.

Maltose and lactose. Citrate-NaCl-NaCl-NaCl and citrate-NaCl-NaCl-(NaCl-sugar) suspensions were allowed to stand four hours before use. From figures 2 and 3 it can be seen that as increasing quantities of maltose or lactose are added to the system as component (a) there occurs a slight increase of resistance. As increasing quantities of maltose or lactose are added via the red cell suspension, there is a further increase in resistance. These sugars differ from the others used in that their prolonged effect is a relative increase of resistance.

Levulose and rhamnose. Citrate-NaCl-NaCl-NaCl, citrate-NaCl-(NaCl-levulose)-(NaCl-levulose), and (citrate-NaCl)-NaCl-NaCl-(NaCl-rhamnose) suspensions were kept for three hours before use. The immediate effect of levulose or rhamnose is an increase in resistance; the prolonged effect is an actual decrease of resistance.

Dextrose and galactose. Citrate-NaCl-NaCl-(NaCl-sugar) suspensions were kept for three hours before use. The immediate effect of galactose and usually of dextrose, is a decrease of resistance. The prolonged effect of either sugar is a further decrease of resistance. Somewhat variable results have been obtained with dextrose, especially with different samples of the sugar; the immediate effect is usually one of decreased resistance but at times an increase of resistance is obtained when the sugar is put into the system as component (a), as shown in figure 6.

(b) The following experiments show differences in the resistance of hypotonic systems produced by the penetration of osmotically active substances through the cell membrane.

Experiment I. A 0.5 standard citrate-NaCl-NaCl-NaCl suspension of red cells (man) was prepared and used in the formation of the following systems at 25°C.:

(a)	(b)
0.05 cc. 0.586 per cent NaCl	0.05 cc. 1.152 per cent urea
—— cc. 0.80 per cent NaCl	—— cc. 0.80 per cent NaCl
—— cc. distilled water	—— cc. distilled water
0.40 cc. cell suspension	0.40 cc. cell suspension

The time of hemolysis (incomplete) was measured with a stop-watch.

Initial tonicity of system	*Time of hemolysis* (a)	(b)
0.32 per cent	0.4 mins.	0.4 mins.
0.34 per cent	0.5 mins.	0.5 mins.
0.36 per cent	0.6 mins.	0.65 mins.
0.38 per cent	1.0 mins.	0.75 mins.
0.40 per cent	more than 3.0 mins.	1.3 mins.

Resistance of (a) = 0.37 per cent; of (b) = 0.39 per cent.

Experiment II. Two standard citrate-NaCl-NaCl-(mixture) suspensions of red cells (man) were prepared. Suspension (a): cells from 0.5 cc. of blood suspended in a mixture of 8 cc. of 0.80 per cent NaCl and 2 cc. of 0.586 per cent NaCl. Suspension

(b): cells from 0.5 cc. of blood suspended in a mixture of 8 cc. of 0.80 per cent NaCl and 2 cc. of 1.152 per cent urea. The following systems were formed:

(a)	(b)
—— cc. 0.80 per cent NaCl	—— cc. 0.80 per cent NaCl
—— cc. distilled water	—— cc. distilled water
0.40 cc. ½ standard suspension	0.40 cc. ½ standard suspension

Before adding suspensions (a) and (b) to systems (a) and (b), respectively, they were subjected to the following treatments: 0.40 cc. of the original standard suspension was squirted violently into a test tube containing its respective mixture (given below) and 0.40 cc. of the new 0.5 standard suspension thus formed added immediately and quickly to the system. The mixtures used to dilute suspensions (a) and (b) are:

(a)	(b)
0.08 cc. of 1.152 per cent urea	0.08 cc. of 0.586 per cent NaCl
0.32 cc. of 0.80 per cent NaCl	0.32 cc. of 0.80 per cent NaCl

This experiment was done at 25°C. and the time of hemolysis obtained with a stopwatch. Results:

Initial tonicity of system	*Time of hemolysis* (a)	(b)
0.32 per cent	0.4 mins.	0.5 mins.
0.34 per cent	0.5 mins.	0.5 mins.
0.36 per cent	0.5 mins.	0.6 mins.
0.38 per cent	0.65 mins.	0.85 mins.
0.40 per cent	1.1 mins.	more than 3.5 mins.

Resistance of (a) = 0.39 per cent; of (b) = 0.37 per cent. Systems (a) and (b) have approximately equal extracellular concentrations of urea.

Experiment III. Standard citrate-NaCl-NaCl-NaCl suspensions of the red cells of man prepared. Suspension (a): cells in 0.60 per cent NaCl; suspension (b): cells in 0.70 per cent NaCl; both allowed to stand for three hours. The systems were formed:

(a)	(b)
—— cc. 0.80 per cent NaCl	—— cc. 0.80 per cent NaCl
—— cc. distilled water	—— cc. distilled water
0.40 cc. cell suspension (½ standard)	0.40 cc. cell suspension (½ standard)

Forty hundredths cubic centimeter of the standard suspension (a) was quickly mixed with 0.40 cc. of 1.0 per cent NaCl and 0.40 cc. of the 0.5 standard suspension thus formed added to system (a). In the same way, equal volumes of suspension (b) and 0.9 per cent NaCl were mixed and 0.40 cc. of the mixture added at once to the system (b). Time of hemolysis was obtained with a stopwatch. Results:

Initial tonicity of system	*Time of hemolysis* (a)	(b)
0.32 per cent	0.45 mins.	0.45 mins.
0.34 per cent	0.45 mins.	0.45 mins.
0.36 per cent	0.60 mins.	0.70 mins.
0.38 per cent	0.85 mins.	1.0 mins.
0.36 per cent	0.65 mins.	0.75 mins.
0.38 per cent	0.75 mins.	0.90 mins.
0.40 per cent	1.35 mins.	1.80 mins.
0.42 per cent	2.70 mins.	more than 4.0 mins.

Resistance of (a) is less than resistance of (b).

DISCUSSION AND CONCLUSIONS. These experiments show that the addition of a sugar solution to a hypotonic system produces a change in the resistance of the system, that within certain limits the greater the quantity of sugar solution in the system the greater the change of resistance and that the magnitude of the resistance change is dependent to some extent upon the length of time the red cells remain in contact with the sugar solution before the occurrence of hemolysis. The question arises as to whether the alteration of resistance is due to the addition of a quantity of sugar solution, to the accompanying removal of an equal quantity of saline or to both. The above experiments indicate that the resistance change is not due merely to removal of NaCl from the system, since different sugar solutions affect resistance in different ways and to different extents. There is reason, moreover, to think that the resistance effect is due both to addition of sugar and removal of saline solution, for experiments of the type described indicate that changes of resistance are produced by the addition of various electrolytes as well as by sugars. Ashby (6) and Brooks (7) have shown, also, that contact between red cell and various electrolytes alters the resistance of the cell to hypotonic solutions. Measurements of depression of freezing point of mixtures of saline and M/3.6 sugar solutions have shown no appreciable osmotic changes due to mixing. This would seem to indicate that the changes of resistance obtained in the above experiment are due to changes occurring in the red cell itself.

It is well known that the erythrocyte is easily affected by changes in its suspension medium. The non-nucleated, mammalian red cell has been considered by many investigators to be a balloon-like structure. There is evidence that it possesses a deformable membrane (8), (9), and that its shape may be dependent to a large extent upon the properties of the medium in which it is placed (9)[1] as well as upon its proximity to certain surfaces (10). It has generally been considered that the red cell of man is impermeable to sodium and certain other cations (11) and that its destruction by hypotonic solutions is due to the entrance of water into the cell causing the latter to swell and, eventually, to burst (12).

Although the entrance of water into the mammalian erythrocyte is probably a chief factor, the above experiments indicate that entrance of water is not the only factor that must be considered in an adequate explanation of hypotonic hemolysis. Among other factors deserving consideration are the rate of passage of osmotically active substances through the cell membrane, the mechanical resistance of the cell to increase of intracellular pressure, and the independently occurring changes in the intra-

[1] In the present experiments it has been noted that, while the red cells in saline assume a crenated spheroid or crenated disc-like form, in certain sugar solutions, containing minimum amounts of saline, nearly all of them are cup-shaped.

cellular concentration of osmotically active substances (as, for example, might occur due to changes in dissociation or in the relative amounts of "free" and "bound" water in the cell). If the rate of entrance of water or other penetrating fluid is equal to the rate of decrease of osmotic concentration within the cell, no change of pressure would thereby result inside the cell, and, hence, no hemolysis would occur, unless the penetrating substance should have a direct hemolytic action on the red cell membrane. In the latter case the resulting hemolysis would not be hypotonic in nature but would be lysis resulting from the direct action of a hemolysin, in the sense that saponin and sodium taurocholate are hemolysins having a direct action on the red cell. The other extreme would be reached when the rate of decrease of osmotic concentration within the cell is equal to zero; neglecting other factors, the time of hemolysis would be determined then by only the rate of entrance of water into the cell, the condition assumed by Jacobs (13) in his analysis of hypotonic hemolysis. Between these extremes fall those substances which penetrate the red cell membrane, but which do so at a lesser rate than the rate of entrance of water.

The experiments described under results (b) illustrate the necessity of considering the passage of other substances than water through the red cell membrane during hypotonic hemolysis. Experiment I shows the effect upon resistance of replacing a portion of the NaCl of the system by urea, the replacement being equivalent to increasing the rate of entrance of NaCl into the cell. In this experiment, the urea is added as component (a) and, at the formation of the system, is present in greater concentration outside than inside the cell.

If the urea of system (b) were initially present in greater concentration inside than outside the red cell, the resistance of the system should alter in the opposite way, due to the exit of urea from the cell, system (b) showing not a lesser but a greater resistance than (a). This is shown by experiment II.

If urea penetrates the red cell in either direction, producing the results just described, it is not unlikely that other osmotically active substances may do the same; even those substances to which the red cell is usually considered to be entirely impermeable may penetrate slowly or to only a slight degree (11), (14). A very slow or very slight exit of osmotically active substances from the red cell may appreciably alter the resistance of a hypotonic system, especially when the rate of entrance of water into the cell is low and when there is only a small difference between internal osmotic pressure and the maximum resistance of the cell to such pressure. It has been noted by Jacobs (13) that under conditions similar to these it is sometimes difficult to obtain the expected measurement of hemolysis. Experiment III shows that the resistance of a pure NaCl system may be appreciably affected by slight intracellular changes in the concentration.

of osmotically active substances. The results can be satisfactorily explained on the assumption that as water enters the cell certain substances effectively leave the cell.

The latter experiments as well as those with sugars show that the resistance of a hypotonic hemolytic system is not determined solely by the rate of entrance of water into the cell but by other factors as well. The latter experiments show that in certain hypotonic systems it is necessary to consider the possibility of passage of osmotically active substances as well as water through the red cell membrane; this is in agreement with the recent conclusions of Kerr (11), of Ponder and Saslow (14) and of others that the impermeability of the red cell for certain cations is less complete than it is usually thought to be. Further work is necessary in order to determine the manner in which a change of resistance is produced by the presence of sugar solution in the hypotonic system.

SUMMARY

1. M/3.6 solutions of the sugars sucrose, levulose, rhamnose, maltose, lactose, and, in general, dextrose, produce an increase in the resistance of the hemolytic system, when added to the system as component (a).

2. M/3.6 solutions of galactose and some samples of dextrose, produce a decrease in the resistance of the hemolytic system, when added as component (a).

3. Sucrose, levulose, rhamnose, dextrose, and galactose produce a relative decrease in the resistance of the hemolytic system, when added as a portion of the red cell suspension medium, as compared to the increase or decrease of resistance, produced when solutions of these sugars are added as component (a).

4. Maltose and lactose produce a relative increase in the resistance of the hemolytic system, when added as a portion of the suspension medium, as compared to the increase of resistance produced when they are added as component (a).

5. Within limits, the greater the quantity of sugar added to the hemolytic system the greater the immediate or the prolonged effect that is produced.

6. A number of experiments are described which show that changes other than the entrance of water into the red cell occur in the process of hypotonic hemolysis.

BIBLIOGRAPHY

(1) Yeager, J. F. Quart. Journ. Exp. Physiol., 1929, xix, 219.
(2) Ponder, E. Biochem. Journ., 1927, xxi, 56.
(3) Kerr, S. E. Journ. Biol. Chem., 1929, lxxxv, 47.
(4) Ponder, E. Proc. Roy. Soc. B, 1921, xcii, 285.

(5) PONDER, E. AND J. F. YEAGER. Proc. Roy. Soc., 1930, cvi, 506.
(6) ASHBY, W. This Journal, 1924, lxviii, 132 (etc.).
(7) BROOKS, S. C. Journ. Gen. Physiol., 1925, vii, 587.
(8) PONDER, E. Proc. Roy. Soc., B, 1924, xcvii, 138.
(9) GOUGH, A. Biochem. Journ., 1924, xviii, 202.
(10) PONDER, E. Brit. Journ. Exp. Biol., 1928–29, vi, 387.
(11) KERR, S. E. Journ. Biol. Chem., 1926, lxvii, 689, 721.
(12) JACOBS, M. H. Biol. Bull., 1930, lvii, 104.
(13) JACOBS, M. H. Amer. Nat., 1928, lxii, 289.
(14) PONDER, E. AND G. SASLOW. Journ. Physiol., 1930, lxx, 169.

STUDIES ON THE ILEO-CECAL SPHINCTER OF THE DOG

JOSEPHINE HINRICHSEN AND A. C. IVY

From the Departments of Zoology and Physiology, Northwestern University

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The ileo-cecal junction is interesting for three chief reasons: first, because its physiology is, as yet, not completely understood; second, it separates two portions of the alimentary tract which are anatomically and physiologically different; and third, because of the important rôle it is thought to and may play in pathological conditions both of the digestive tract itself and of the body in general. An understanding of the ileo-cecal sphincter may possibly be of as great significance as our knowledge of the pyloric sphincter has proven to be.

The work presented in this paper was undertaken because no attempt at a systematic study of the innervation of the sphincter in the dog has been made, because the effect of distention of various portions of the gastro-intestinal tract in the dog has not been studied completely, because it was thought desirable to repeat in part the work of Toennis (1924, 1928) on dogs with fistulas of the cecum and ileum for the purpose of further studying the control of the sphincter and of correlating such findings with those reported in man, because the activity of the distal ileum during fasting and feeding has not been studied in sufficient detail in the dog, and because such knowledge was necessary as a background for certain contemplated studies on the pathologic physiology of the ileo-cecal sphincter.

We shall not review the anatomical, physiological and clinical literature on the ileo-cecal sphincter, since this has been done rather completely by Alvarez (1928), who has also discussed the possible relation of this sphincter to the problem of intestine autointoxication (1924).

METHODS. Three methods were used in a study of the innervation and normal control of the sphincter in the dog: *a*, In forty acute experiments in which barbital anesthesia was given, nerves were electrically stimulated to determine the extrinsic innervation of the sphincter. In other experiments various portions of the intestine were distended as a means of studying the reflex innervation. Epinephrine and pilocarpine were injected intravenously. *b*. Direct observation of the action of the sphincter on animals with a fistula of the cecum about one inch distal to the sphincter. *c*. Fluoroscopic examination with observation on the response to feeding in dogs with a fistula of the ileum.

The ileo-cecal junction in the dog is marked by a definite sphincteric ring which can be seen on anatomical examination and which can be felt to contract when a finger is inserted into its lumen. The ileo-cecal mesentery is very short and thus brings the sphincter into close anatomical relationship with the celiac and superior mesenteric ganglia.

In the acute experiments contractions were recorded by a balloon, placed in the sphincter, which was so constructed as to be affected only by pressure changes due to contraction of the sphincter and not by peristalsis of the lower ileum and upper colon. The balloon was connected through narrow rubber tubing with a recording tambour and distended with approximately 2 cc. of air. Intact nerves as well as the central and peripheral ends were stimulated: the vagi in the neck, on the esophagus and on the stomach at the cardia, the splanchnics at the crura of the diaphragm, the pelvic nerves at their origin in the sacral region as well as peripheral to the pelvic ganglion. The hypogastric nerve and fibers from the superior mesenteric ganglion accompanying the superior mesenteric blood vessels were also stimulated. Balloons were placed in the intestine and were distended with water at 39°C. Blood pressure tracings were made in most of the experiments. Barbital anesthesia was used. (See fig. 11.)

I. Results on nerve stimulation and distention of the stomach and intestine. *Stimulation of peripheral vagus in the neck:* Elliott (1904), working on the cat, observed only contractions of the sphincter which he attributed to anemia caused by the fall in blood pressure, since he stimulated the vagus in the neck.

In our experiments on the dog the predominating effect of peripheral vagus stimulation (23 times out of 47 stimulations) was diphasic in that a period of slight relaxation or inhibition occurred which was followed by a contraction, the contraction being more evident on the tracing than the relaxation. This was true of either right or left vagus. When the sphincter was manifesting a tonus rhythm, stimulation of the peripheral vagus quite uniformly caused inhibition of the tonus rhythm prior to the subsequent contraction. This occurred even when there was no relaxation of the sphincter prior to contraction. In two experiments only relaxation and in fifteen only contraction occurred. No response was obtained in seven instances. In two cases the blood pressure was not affected by stimulation of the vagus; yet the sphincter first relaxed and then contracted.

Vagus in neck stimulated with sphincter contracted by distention of the duodenum: Stimulation of the peripheral vagus in the neck when the sphincter was in a state of contraction due to distending the duodenum caused only relaxation of the sphincter in three experiments and further contraction in one.

Vagus on the esophagus: In order to avoid stimulation of the cardio-

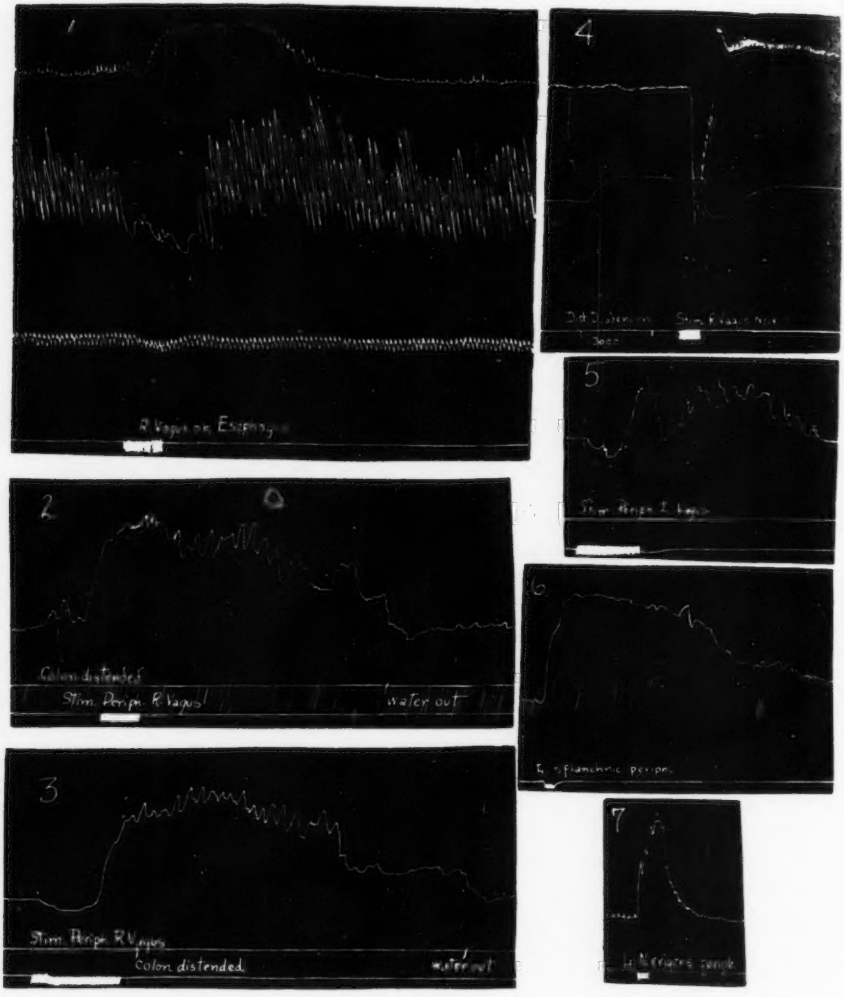

Fig. 1. The upper tracing is made by the ileo-cecal valve, the middle by the pyloric sphincter and the lower by the blood pressure. It is to be noted that stimulation of the right vagus peripherally on the esophagus caused an inhibition of the tonus rhythm of both the ileo-cecal and pyloric sphincters and slight relaxation, which was followed by contraction.

Fig. 2. This tracing demonstrates a contraction of the ileo-cecal sphincter caused by distention of the colon and that stimulation of the peripheral right vagus caused some relaxation.

inhibitory fibers and to rule out the effect of blood pressure, the vagi were stimulated on the esophagus. In six instances contraction only occurred, in two relaxation and in two relaxation followed by contraction. No response occurred in four.

We believe this to be a much better experiment to rule out the effects of the fall in blood pressure than the atropine experiments of Elliott (1904), since atropine may and probably does paralyze the vagus fibers to the sphincter.

Stimulation of central end of vagus: Stimulation of the central end of one vagus caused either contraction (6 times) of the sphincter, or relaxation followed by a contraction (6 times), or a relaxation (2 times). No response occurred in ten instances. Relaxation occurred in one experiment in which both vagi were cut, which indicates that the relaxation was due either to inhibitory impulses via the splanchnics, or to a decrease in the motor tonic action of the splanchnics, or to inhibitory impulses via the pelvic nerves. We believe the most likely explanation to be that splanchnic tone was decreased.

Stimulation of vagi to note the simultaneous effect on the ileo-cecal and pyloric sphincters: It is possible that when the vagus is stimulated, the resulting changes in the stomach secondarily or reflexly excite the ileo-cecal sphincter. In order to note the effect of vagus stimulation on both the pyloric and ileo-cecal sphincters simultaneously in the same animal, an appropriately constructed balloon was placed in the pyloric sphincter and connected with a water manometer so that the action of both sphincters could be observed when the vagi in the neck were stimulated. Stimulation of the peripheral vagi in the neck usually caused preliminary inhibition of the pyloric sphincter which was followed by a rise in tone or a contraction. The pylorus manifested only relaxation in ten out of twenty-three stimulations. The peripheral esophageal vagus was stimulated fifteen times. In seven cases contraction occurred, in seven relaxation, and in one no response was observed. The ileo-cecal sphincter contracted in all these experiments.

Fig. 3. This tracing demonstrates a relaxation of the ileo-cecal sphincter followed by contraction on stimulation of the peripheral right vagus and that distention of the colon had no effect on the response except possibly prolonging it.

Fig. 4. In this figure the upper tracing is the blood pressure record and the middle tracing is from the ileo-cecal sphincter. Note that distention of the duodenum caused the sphincter to contract and that stimulation of the vagus caused relaxation.

Fig. 5. This tracing shows "the relaxation followed by contraction" which occurred rather commonly after stimulation of the peripheral vagus.

Fig. 6. This tracing shows the contraction of the ileo-cecal sphincter resulting from peripheral stimulation of the left splanchnic.

Fig. 7. This tracing shows a contraction of the sphincter on stimulation of the peripheral left pelvic nerve.

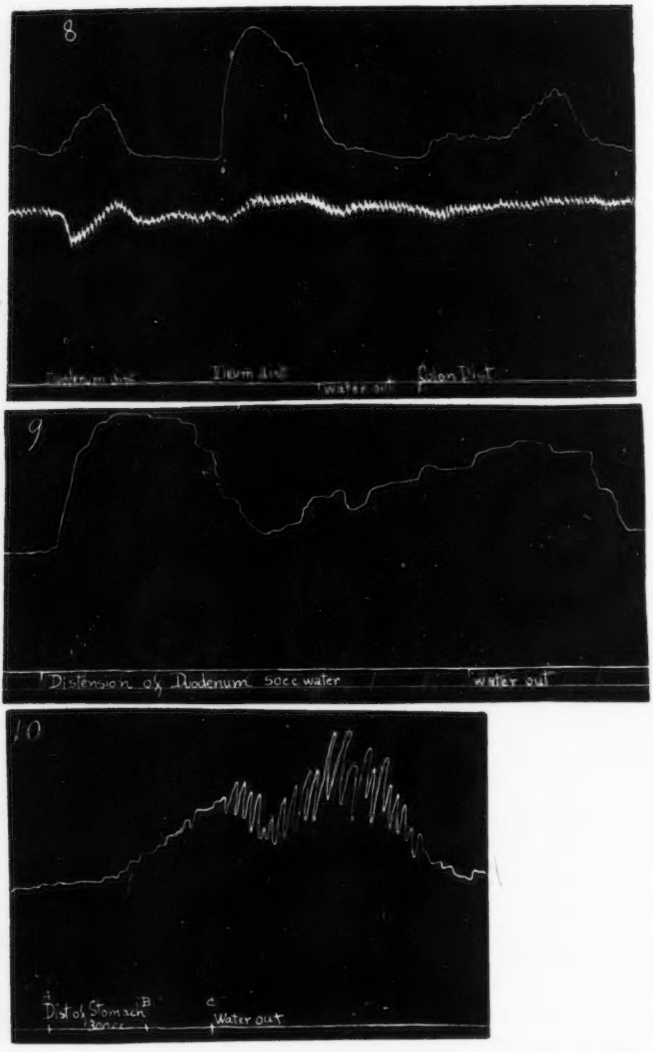

Fig. 8. This tracing shows contractions of the ileo-cecal valve resulting from distention of the duodenum, ileum and colon, successively, in the same dog.

Fig. 9. This tracing shows the response of the ileo-cecal sphincter on distention of the duodenum. The additional or sustained contraction on withdrawal of the distention which sometimes occurred, is to be noted.

Fig. 10. This tracing shows the response of the ileo-cecal sphincter on distention of the stomach. The additional or sustained contraction after withdrawal of the distention, which sometimes occurred, is to be noted.

The ileo-cecal sphincter responds as quickly to vagus stimulation as does the pyloric sphincter and the stomach, which leads us to believe that the ileo-cecal sphincter is directly controlled by the vagus through the celiac or superior mesenteric ganglion.

Stimulation of the gastric vagi: Stimulation of the vagi on the stomach near the cardia had no effect on the ileo-cecal sphincter in eight experiments. This shows that the stomach may contract without influencing the ileo-cecal sphincter and supports the preceding opinion.

Stimulation of the splanchnic nerves: Marked visceral and skeletal activity followed stimulation of the intact splanchnic nerves, which was associated with contraction of the sphincter. The sphincter contracted markedly when the peripheral ends and less markedly when the central ends were stimulated. Of twenty-three stimulations of the peripheral ends, twenty resulted in contraction, three were negative and in no case was relaxation produced.

Stimulation of the central ends of the splanchnic nerves caused contraction in all but two instances (17 stimulations). Relaxation in one experiment resulted when both vagi and splanchnics were cut and was the result of stimulating the central end of the left splanchnic nerve. Stimulation of the central end of the right splanchnic in this instance was ineffective. In this experiment relaxation could have been due only to inhibitory influences acting via the pelvic nerves or to a decrease in tonic control mediated via the unsectioned lumbar sympathetic nerves. In the other experiment in which relaxation occurred, as well as in all other experiments on splanchnic stimulation, both vagi or one vagus nerve were intact.

Stimulation of peripheral splanchnic with sphincter reflexly contracted: Stimulation of the peripheral ends of the splanchnic nerves when the sphincter was contracted due to distention of the ileum increased the contraction, and in no instance decreased the tone or actually caused relaxation of the sphincter (6 experiments).

Simultaneous stimulation of splanchnic and vagus nerves: Stimulation of the peripheral splanchnic and vagus nerves simultaneously resulted in marked contraction (4 experiments). The vagus failed to exercise an antagonistic action as far as could be determined from the resulting tracings.

Superior mesenteric ganglion: Fibers from the superior mesenteric ganglion, along the superior mesenteric artery, and fibers going to the inferior mesenteric ganglion when stimulated caused the sphincter to contract every time.

Hypogastric nerves: Stimulation of the peripheral hypogastric nerve resulted in contraction of the sphincter in twenty-five of thirty experiments. This was, however, invariably accompanied by strong contraction

of the colon or cecum. For this reason the extent of the direct motor effect on the sphincter itself is somewhat obscure, since the contracting colon may have reflexly or secondarily caused the contraction of the sphincter.

Stimulation of the central end of the hypogastric nerve also caused contraction without a uniform effect on the colon. Contraction occurred in fourteen out of fifteen experiments, relaxation occurring each time. This occurred whether the vagi were cut or intact.

Pelvic nerves: The pelvic nerves were stimulated near their origin in the sacral region. Visceral and skeletal movements were produced when the intact nerves were stimulated. Stimulation of both central and peripheral ends caused definite contractions of the sphincter, some being quite delayed and others occurring immediately. The colon, of course, always contracted. When the peripheral end was stimulated, eighteen out of twenty-four experiments resulted in contraction, five being negative and one resulting in relaxation of the sphincter. When the central end was stimulated, twelve out of sixteen experiments resulted in contraction, three being negative and one resulting in relaxation.

Fig. 11

Somatic sensory nerve: Stimulation of the sciatic nerve had no effect on the sphincter, which confirms Elliott (1904).

Distention of the stomach and intestine: The sphincter was reflexly stimulated to contract by distention of various portions of the gut. A balloon was placed in the lumen of the bowel and distended with water at 39°C. Distention of the stomach, duodenum, ileum and colon resulted in contraction of the sphincter; or, if there was no contraction on distending, it often resulted when the pressure was released. The contraction resulting from distention of the stomach was slight and long continued, that from the ileum and duodenum the most marked and generally relatively brief. Of thirty distentions of the stomach, twenty resulted in contraction, and ten produced no change. Of twenty-nine distentions of the duodenum, twenty-two resulted in contraction, and seven produced no change. Of twenty-three distentions of the distal ileum, seventeen resulted in contraction, two in relaxation and no change occurred in four. Of seventeen distentions of the mid-portion of the colon, thirteen resulted in contraction, and no change in four.

Spontaneous contractions: In many of these experiments well marked rhythmic contractions of the sphincter were observed and were best seen at the beginning of the experiment.

Rôle of extrinsic and intrinsic nervous mechanism: The method of distending portions of the gut was made use of in studying the comparative rôle of the extrinsic and intrinsic nervous mechanism. Since distention

of the duodenum or ileum reflexly causes contraction of the sphincter, one would suppose the path of the nerve impulse to be via the splanchnic and vagus nerves, and in case of distention of the colon, via the pelvic and hypogastric nerves, or via the intrinsic enteric nerves. Cutting the extrinsic nerves then should abolish the contraction unless the intrinsic nervous mechanism plays a part.

The following are the results of seven experiments: Only animals could be used in which marked contraction occurred on distention of the gut. It may be noted here that there is a difference in the tone of the sphincter and therefore in the response obtainable in different animals and in the same animal from time to time. In four experiments, sectioning both vagi and splanchnics, but not one or the other, abolished the reflex contraction resulting from distending the duodenum. In three other experiments contraction still followed after the extrinsic nerves supplying the particular loop of gut distended had been cut. In one of these experiments the duodenum, in another the ileum and in a third the colon were the parts distended. In one of these, cutting through the colon, and in another, cutting through the ileum within three inches of the sphincter abolished the reflex. Repeated distention of a denervated portion of the gut (ileum or colon) no longer gave contraction of the sphincter. Distending isolated loops of ileum and colon with extrinsic nerves intact caused contraction of the sphincter on two occasions. It is obvious that on the basis of these results one cannot state the relative importance of the extrinsic and intrinsic nervous mechanism in the control of the sphincter. The results do show, however, that contraction of the sphincter may be brought about via either an intrinsic or an extrinsic nervous mechanism. The observations of Toennis (1924, 1928) cited above show that after an interval of a few days the sphincter can function "normally" when devoid of extrinsic innervation.

Effect of drugs: Epinephrine 0.1 cc. 1:10,000 injected into the femoral vein caused marked contraction, the contraction curve being similar to that obtained from stimulation of the splanchnics. Pilocarpine 1–2 cc. of a 1:10,000 solution resulted when injected in a slow rising, long continued contraction.

II. Observation of the action of the sphincter through a cecal fistula. In four dogs a fistula was made in the colon within one inch of the ileo-cecal sphincter. Through this fistula the sphincter could be seen as a contracted ring which, when the ileum was actively discharging, would relax at intervals of from a few seconds to five or ten minutes to allow quantities of from 2 cc. to 10 cc. of material to pass. In three dogs so prepared there was a considerable loss of chyme and fluid. The distal ileum became very active and discharged contents almost continually, the material being in general of a very soft, almost fluid consistency. In a

fourth dog, after a slight disturbance for several days after the operation there resulted a complete adjustment to the new condition. The material passed became more fecal in consistency, therefore there was not a great loss of fluids and the animal remained in good health. The sphincter remained closed except when food was passing. At that time it could be seen to actively relax, contracting again immediately after the passage of contents. Stimulation of the projecting portion of the colon mucosa caused the sphincter to contract tightly. No definite effect of feeding was observable in three of the animals because of irregular appetite and eating habits. The hypermotility of the ileum leads to almost "continuous" evacuation. In the fourth dog in which the appetite was very good, it was noted on many occasions that when a meal was taken the activity of the ileum was reflexly stimulated and material began to pass out of the fistula almost immediately after feeding.

III. Fluoroscopic observation of the action of the ileo-cecal sphincter. Three dogs were prepared as follows in order that the action of the sphincter could be satisfactorily observed by means of the fluoroscope. A loop of ileum, four inches long and approximately fifteen inches proximal to the ileo-cecal sphincter was brought out of the peritoneal cavity and fixed directly beneath the skin. After the incision had completely healed, a small opening was made through the skin into the ileal loop. This opening was only large enough to allow the passage of a very fine rubber tube. Through this tube a barium milk mixture consisting of 10 grams of barium sulphate and 15 cc. of milk (39°C.) was slowly injected while the animal was being fluoroscoped. (The animals were fasted 24 to 48 hours.) The mass could be seen to pass into the ileum and to be gradually moved toward the sphincter. Almost invariably a small amount of the material would immediately pass through the ileo-cecal sphincter. After that the sphincter would close tightly, allowing no further passage. Active rhythmic segmentation, longitudinal shortening, peristalsis, and a "churning effect" could be seen in the ileum. This would continue for long periods of time, on the average of from $\frac{1}{2}$ to $1\frac{1}{2}$ hours, without any relaxation of the sphincter occurring. If at this time the animal was given a full meal consisting of raw, ground meat, milk and bread (the animal ate while being observed) there occurred in most instances a gastro-ileal or duodenal-ileal reflex. This reflex caused the ileal loops to become very active. The sphincter relaxed before an advancing wave of contraction and there was a rapid passage of material into the colon, almost but not completely emptying the small intestine within fifteen minutes after the animal had started to eat.

The reflex is not a psychic one in the dog because the sight and smell of food or even the eating of a very small amount of food at no time was sufficient to affect the activity of the ileum. The reflex was most marked

when the animal was very hungry and was not obtained if the dog had been recently fed. For that reason the observations were made 24 to 48 hours after the last meal. Out of twenty-one observations the "feeding reflex" occurred in thirteen cases. The other eight were negative or doubtful, in that complete or definite emptying did not result within fifteen minutes.

DISCUSSION. *Vagus action:* It is evident from our results on the effect of stimulation of the vagus that in the dog it contains both motor and inhibitory fibers for the ileo-cecal sphincter and that these fibers leave the vagus above the stomach. It is impossible to state whether the predominating action of the vagus is motor or inhibitory but the results on the effect of stimulation of the vagus when the sphincter is contracted by reflex stimulation show that the vagus in the dog can inhibit the contracted sphincter. The chief inhibitory effect is on the tonus rhythm. It is possible in the instances in which only relaxation occurred, that the sphincter was hypertonic and in the instances in which only contraction occurred that the sphincter was hypotonic. We cannot make an absolute statement on this point, since our balloon was so small by necessity that accurate determination of amounts of pressure change from animal to animal was not possible.

The tracing that one obtains when vagus stimulation causes relaxation or inhibition of the tonus rhythm followed by contraction is similar to the tracing one obtains from the pyloric sphincter and also from the cardiac sphincter when a wave of primary peristalsis of the esophagus affects this latter sphincter.

Our results on the simultaneous effect of vagus stimulation on the pyloric and ileo-cecal sphincters and on stimulating the gastric vagi show that the stomach may contract without affecting the ileo-cecal sphincter and indicate that the vagi exert a direct control over this sphincter via either the celiac or superior mesenteric ganglion.

This effect of the vagus on the ileo-cecal sphincter falls in line with the observations of Thomas and Wheelon (1922) and Carlson and Litt (1924) on the pyloric sphincter. However, we believe that the normal response to vagus stimulation or the normal vagal control of the cardiac, pyloric and ileo-cecal sphincters is to produce relaxation or inhibition of the tonus rhythm followed by contraction. This view is supported by the well-known relation of the cardiac sphincter, the pyloric sphincter and ileo-cecal sphincter to peristalsis in the esophagus, in the pyloric antrum and distal ileum, respectively.

Splanchnic action: The findings of Elliott (1904) on the effect of splanchnic stimulation in the cat are also true for the dog. Contraction of the sphincter occurred uniformly, relaxation not being observed in twenty-three instances. It is, therefore, not likely that the splanchnic

nerves carry inhibitory fibers to the ileo-cecal sphincter. This is further evidenced by the fact that stimulation of the splanchnics when the sphincter is in a state of contraction induced reflexly or directly does not cause relaxation. It should be noted, however, that in one experiment with both vagi cut, stimulation of the central end of one vagus caused relaxation of the sphincter, which might have been due either to inhibitory impulses via the splanchnics, or, as we prefer, to reflex decrease in splanchnic motor tone.

It is not surprising that Hannes (1920) found that fasting decreases the tone of the sphincter, since in hunger vagal motor tone increases unquestionably which may lead to a decrease in sphincteric tone. In support of this it is known that hunger increases the rate of emptying of the stomach (Fauley and Ivy, 1929) probably because of an increase in gastric tone and a decrease in sphincteric tone.

The fact that the splanchnics exercise a tonic motor action on the sphincter and that their section leads to incompetence according to both Elliott and Toennis, which is confirmed by us, may be of considerable practical significance. A decrease in its tone may account for the regurgitation found in some human subjects, and the section or trauma of the adjacent nerves in the mesentery of the ileum during an appendectomy may account in part for the occasional post-operative intussusception of the ileum into the cecum.

Action of superior mesenteric nerve fibers: Our findings on the dog on stimulating fibers going to and from the superior mesenteric ganglion confirm those of Elliott on the cat.

Hypogastric action: Although we found that stimulation of the hypogastric nerves both peripheral and central caused the sphincter to contract, we cannot be certain that the contraction was due to the direct stimulation of efferent motor nerves, because of the vigorous contractions of the colon resulting when the peripheral ends were stimulated. All we can say is that a contraction occurred, but we were at a loss to devise experiments to determine the mechanism without a mutilating operation.

Pelvic nerve action: Elliott observed in only one of his experiments in the cat that stimulation of the pelvic nerve (n. erigens) caused a delayed contraction of the sphincter which he interpreted as being secondary to the contraction of the colon. Some of the contractions we observed were delayed but others occurred quite soon after the stimulation. However, we prefer the interpretation of Elliott to explain our results, since the question cannot be answered without a mutilating operation.

Stimulation of central end of autonomic nerves: The results on the stimulation of the central end of the vagi, splanchnics and pelvic nerves show that the sphincter can be influenced reflexly by afferent impulses passing into the cord and brain via any of these nerves.

Sciatic nerve: It is of interest to note that the ileo-cecal sphincter of the

dog failed to respond to excitation of the central end of the sciatic nerve as was observed by Elliott in the cat, since in this respect this sphincter does not fall in line with the pyloric sphincter which according to Carlson and Litt (1924) may contract or relax on stimulation of the sciatic.

The extrinsic and intrinsic nervous mechanism: The fact that by distending loops of duodenum, ileum and colon one is able to cause contraction of the sphincter when the extrinsic nerves are cut shows along with the observations of Toennis that the intrinsic nerves play an important rôle in the control of the sphincter. We have made no observations on the mechanism involved in the "feeding reflex."

Drugs: The results on the action of adrenalin and pilocarpine were as might be predicted from the well-known action of the drugs and the innervation of the sphincter.

Observations on the cecal fistula and action of the distal ileum: Our results in general show that the ileo-cecal sphincter stands in the same relation to the colon and small intestine as the pyloric sphincter stands in relation to the stomach and duodenum. Irritation of the mucosa of the cecum in the region of the sphincter causes it to contract or go into spasm and movements of the ileum are related to the activity of the sphincter. The observations on the dog confirm those on man.

The lower ileum may manifest considerable rhythmic segmentation, peristalsis and "churning" movements without evacuating any of its contents, but on an adequate stimulus from the stomach or duodenum the distal ileum becomes more active and the sphincter relaxes before a vigorous advancing wave of contraction and the contents of the distal ileum are discharged into the colon in intermittent gushes. Obviously the sphincteric mechanism must be in some special state before a peristalsis will cause it to relax and permit chyme to pass, in which respect it is analogous to the pyloric sphincter.

The gastro-ileal reflex or the duodeno-ileal reflex is as true a reflex as any which is observed in the gastro-intestinal tract, our observations confirming those of Macewen (1904), Hurst (1913), Holzknecht (1909), Barclay (1912), Hurst and Newton (1913) and Case (1914) and Short (1919) on the human subject. We believe that the duodeno-ileal reflex is a more correct term in view of the fact that the changes in the ileo-cecal sphincter are more readily brought about by distention of the duodenum than distention of the stomach which is supported by the observations of Ivy and McIlvain (1923), Welch and Plant (1926) and Pearcy and Van Liere (1926). The fact that distention of the various portions of the gut causes the sphincter to contract is not paradoxical necessarily to the phenomenon of the "feeding reflex" for several reasons: *a*, the reflex mechanism of the two may be different; *b*, the state of the reflex mechanism if the same one is concerned, may be affected in one way by duodenal

motility and in another way by duodenal distention; *c*, the contraction of the sphincter on duodenal distention may place the sphincteric mechanism in such a state that it will relax before an advancing wave in the ileum and then quickly and firmly contract after passage of chyme into the cecum, preventing regurgitation. Other explanations might be offered, but the latter seems most satisfactory to the authors in view of the effect of fasting on the tone of the sphincter and of the function of the sphincter which is to retain the chyme in the ileum until digestion and absorption are accomplished and to prevent regurgitation of fecal material into the ileum.

SUMMARY

1. The ileo-cecal sphincter in the dog is a true physiological sphincter.

2. The vagus nerve contains both motor and inhibitory fibers for the ileo-cecal sphincter. The opinion is expressed that the normal vagal control of the ileo-cecal sphincter, the pyloric and cardiac sphincter results in relaxation followed by contraction.

3. The splanchnic nerves contain only motor fibers for and play a rôle in maintaining the tone of the ileo-cecal sphincter, which confirms Elliott's work on the cat and Toennis' on the dog. It is pointed out that this may explain in part certain clinical observations.

4. Reflex contraction of the sphincter may be brought about through either the extrinsic or intrinsic nervous mechanism.

5. Contraction of the sphincter may be brought about by stimulation of almost any nerve in the abdomen and by the distention of the stomach, duodenum, ileum and colon. Motor disturbances of the ileo-cecal sphincter may thus be brought about by local pathological changes in the ileum and colon, by abnormal states of the nervous system and by irritation of any portion of the gastro-intestinal tract.

6. Stimulation of the sciatic nerve failed to cause a motor change of the ileo-cecal sphincter, confirming Elliott.

7. Adrenalin and pilocarpine cause the sphincter to contract.

8. A "feeding reflex" is a definite physiological phenomenon, excitation of the duodenum being the most potent factor concerned in the elicitation of this reflex.

9. The emptying of the distal ileum is described and observations of the ileo-cecal sphincter under direct vision in dogs with a cecal fistula are recorded.

BIBLIOGRAPHY

ALVAREZ, W. C. 1924. Physiol. Reviews, iv, 352.
1928. The mechanics of the digestive tract, New York.
BARCLAY, A. E. 1912. Arch. Roentg. Ray, xvi, 422.
BAUHINI, C. 1579. Spachius (I) Gynaecorium etc. fol. Argentinae, 480, loc. cit. ELLIOTT, 1904, Journ. Physiol., xxxi, 157.
BEATTIE, J. 1924. Journ. Anat., lix, 56.
BEUTENMUELLER, N. 1920. Muench. Med. Wochenschr., lxvii, 743.
CANNON, W. B. 1911. Arch. Int. Med., viii, 417.
CARLSON, A. J. AND S. LITT. 1924. Arch. Int. Med., xxxiii, 281.
CASE, J. T. 1914. Arch. Roentg. Ray, xix, 45. Proc. 17th Internat. Congr. Med., Sec. 22, Radiology, Pt. 2, 11–41; Med. Record, lxxx, 415.
1915. Penna. Med. Journ., xviii, 683.
ELLIOTT, T. R. 1904. Journ. Physiol., xxxi, 157.
FAULEY, G. B. AND A. C. IVY. 1929. This Journal, xci, 206.
GOOD, J. M. 1822. The study of medicine, loc. cit. KELLOGG, 1913, Med. Record, lxxxiii, 1105.
GROEDEL, F. M. 1912. Die Magenbewegungen, Hamburg.
HANNES, B. 1920. Muench. Med. Wochenschr., lxvii, 745.
HEILE, B. 1905. Mitt. a. d. Grenzgeb. d. Med. u. Chir., xiv, 474.
HOLZKNECHT, G. 1909. Muench. Med. Wochenschr., lvi, 2401.
HUNTINGTON, G. S. 1903. The anatomy of the human peritoneum and abdominal cavity. Philadelphia.
HURST, A. F. 1913. Journ. Physiol., xlvii, 54.
HURST, A. F. AND A. NEWTON. 1913. Journ. Physiol., xlvii, 57.
IVY, A. C. AND G. B. MCILVAIN. 1923. This Journal, lxvii, 124.
JONES, N. W. 1923. Amer. Journ. Med. Sci., clxvi, 710.
KEITH, A. 1903. Journ. Anat. and Physiol., xxxviii, 7.
KELLOGG, J. H. 1913. Med. Record, lxxxiii, 1105. Surg., Gynec. and Obst., xvii, 563.
KURODA, M. 1916. Jour. Pharm. Exper. Therap., ix, 187.
LEBON, H. AND P. AUBOURG. 1913. Presse Med., xxi, 566.
LYMAN, H. 1913. This Journal, xxxii, 61.
MACEWEN, W. 1904. Lancet, ii, 995.
PEARCY, J. F. AND E. J. VAN LIERE. 1926. This Journal, lxxviii, 64.
PLESCH, J. 1928. Zeitschr. f. Klin. Med., cviii, 340.
RUTHERFORD, A. H. 1914. The ileocecal valve. New York.
SHORT, A. R. 1919. Brit. Med. Journ., ii, 164.
THOMAS, J. E. AND H. WHEELON. 1922. Journ. Lab. Clin. Med., vii, 375.
TOENNIS, W. 1924. Pflüger's Arch., cciv, 477.
1928. Arch. Klin. Chir., cli, 785.
WELCH, P. B. AND O. H. PLANT. 1926. Amer. Journ. Med. Sci., clxxii, 261.
WHITE, F. W. 1918. Amer. Journ. Med. Sci., clvi, 184.
WINKLER AND KATZ. Loc. cit. v. PFUNGEN: 1906, Pflüger's Arch., cxiv, 386.

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